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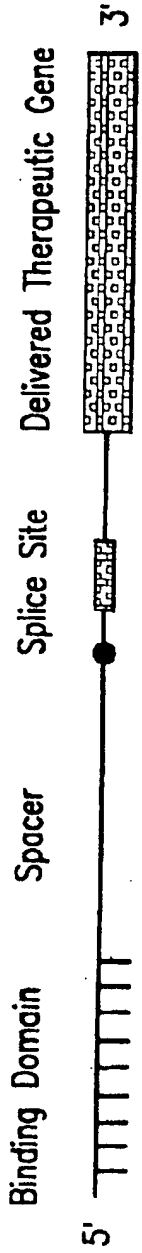


FIG.1A

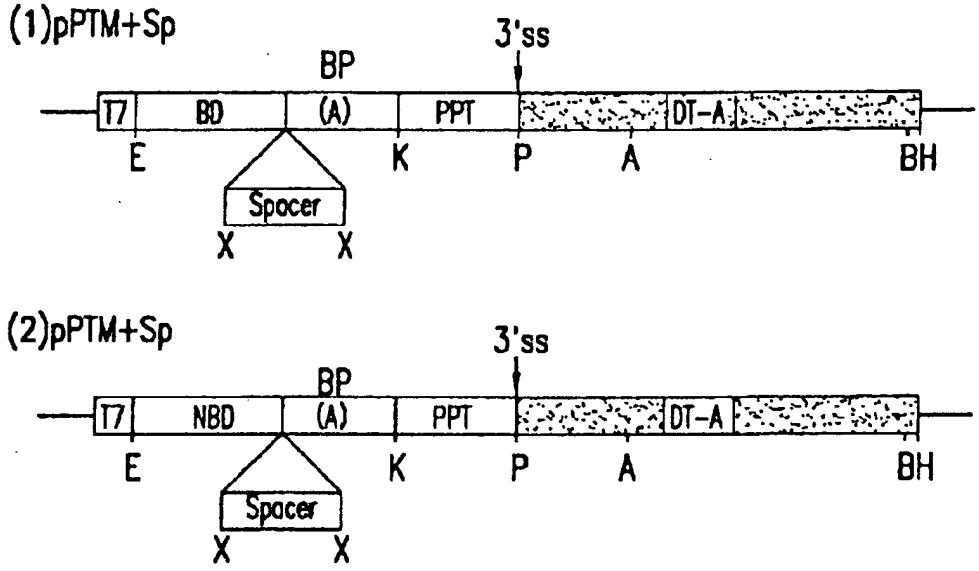

(1)pPTM+Sp
3'ss
BP
T7
BD
(A)
PPT
DT-A
E
K
P
A
BH
Spacer
X
X
(2)pPTM+Sp
3'ss
BP
T7
NBD
(A)
PPT
DT-A
E
K
P
A
BH
Spacer
X
X

FIG.1B

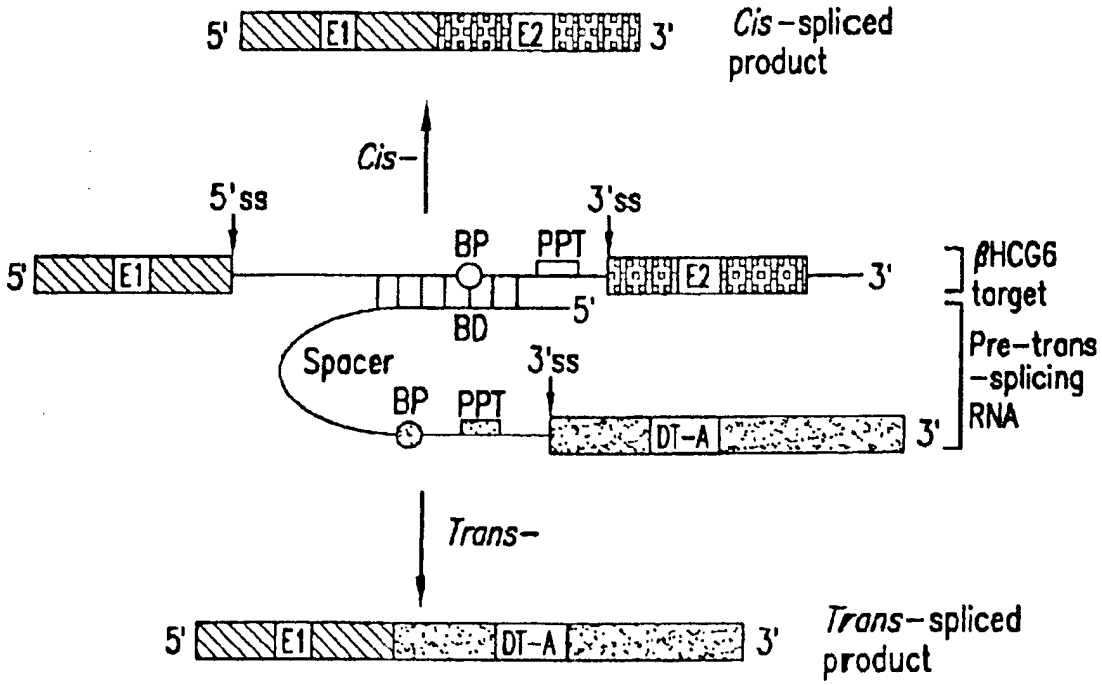

5'
E1
E2
3'
Cis-spliced product
Cis-
5'ss
3'ss
BP
PPT
5'
E1
E2
3'
βHCG6 target
BD
5'
Spacer
3'ss
BP
PPT
DT-A
3'
Pre-trans-splicing RNA
Trans-
5'
E1
DT-A
3'
Trans-spliced product

FIG.1C

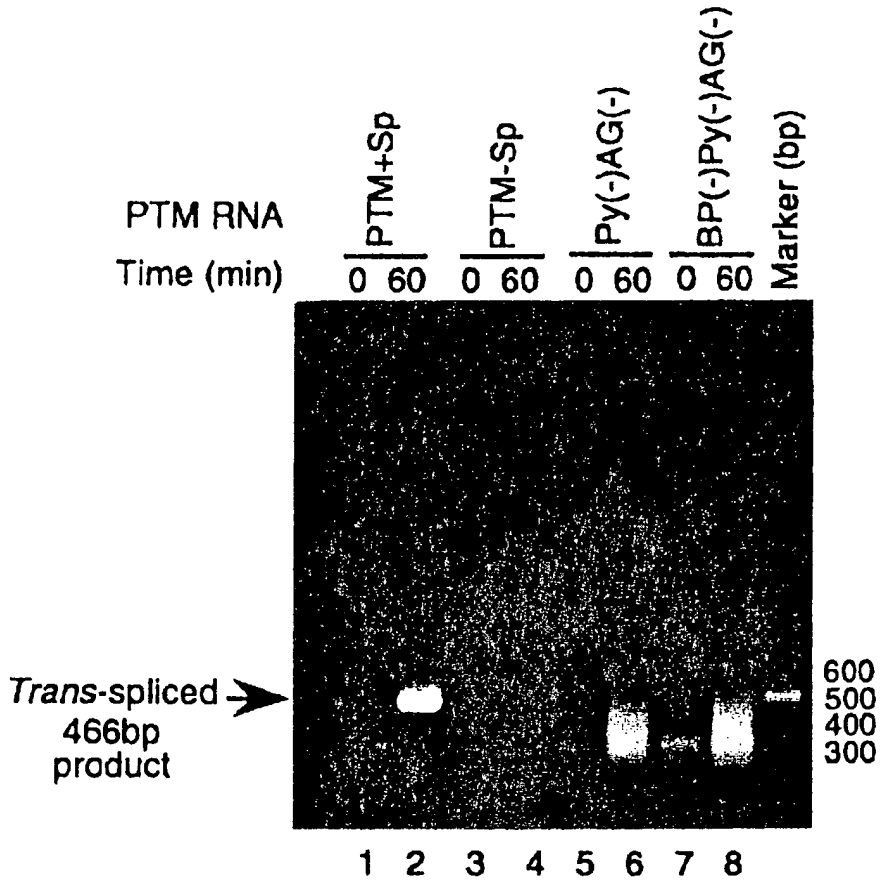

PTM RNA
Time (min)
PTM+Sp
PTM-Sp
Py(-)AG(-)
BP(-)Py(-)AG(-)
Marker (bp)
0 60 0 60 0 60 0 60
Trans-spliced
466bp
product
600
500
400
300
1 2 3 4 5 6 7 8

FIG.2A

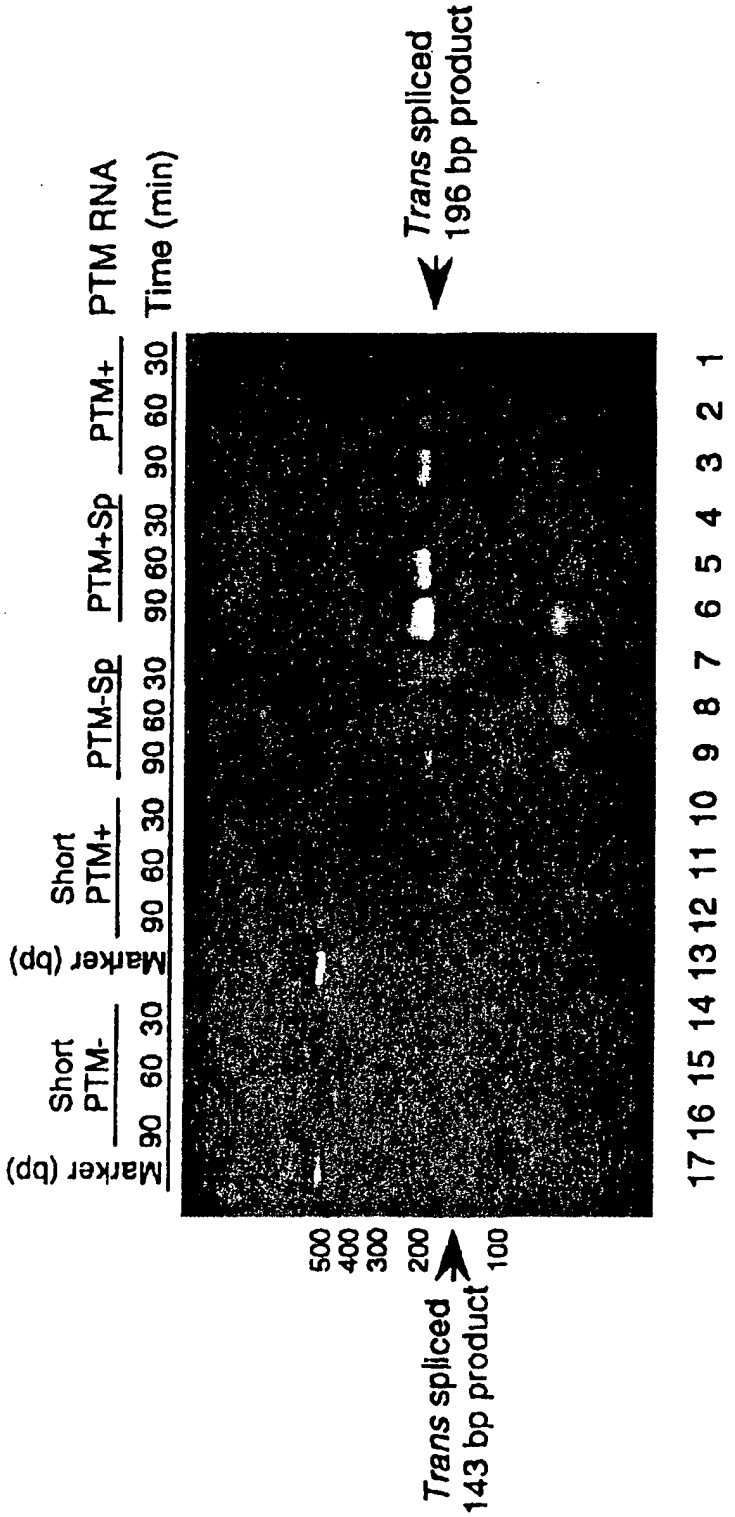

Marker (bp)
Short PTM-
90 60 30
Marker (bp)
Short PTM+
90 60 30
PTM-Sp
90 60 30
PTM+Sp
90 60 30
PTM+
90 60 30
PTM RNA
Time (min)
Trans spliced 196 bp product
500
400
300
200
100
Trans spliced 143 bp product
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

FIG.2B

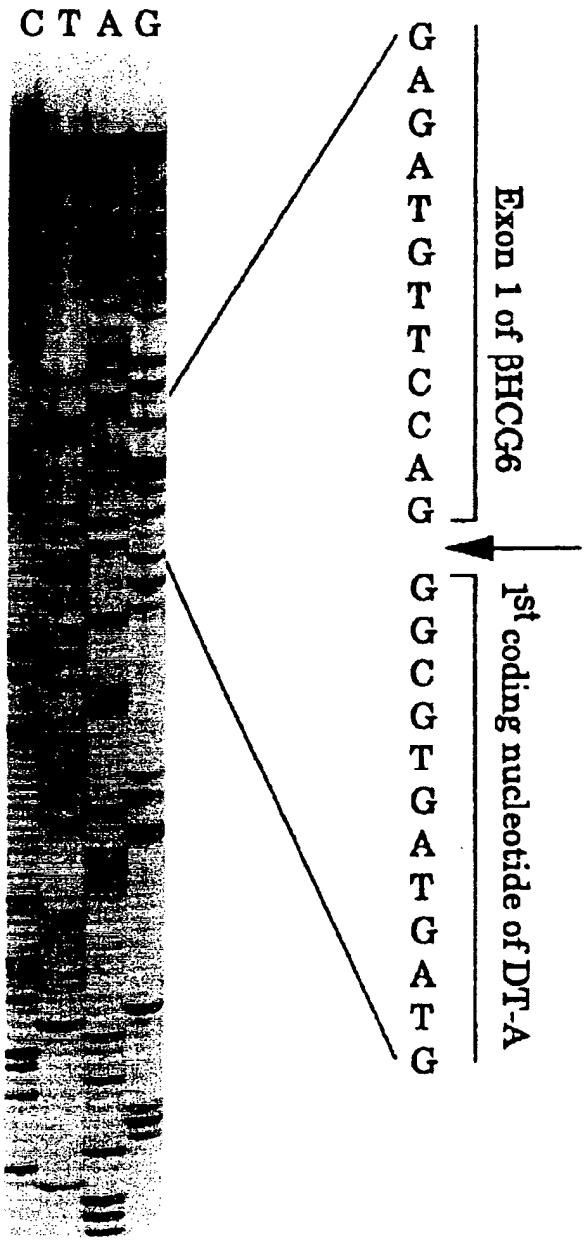

C T A G
G
A
G
A
T
G
T
T
C
C
A
G
Exon 1 of βHCG6
1st coding nucleotide of DT-A
G
G
C
G
T
G
A
T
G
A
T
G

FIG.3

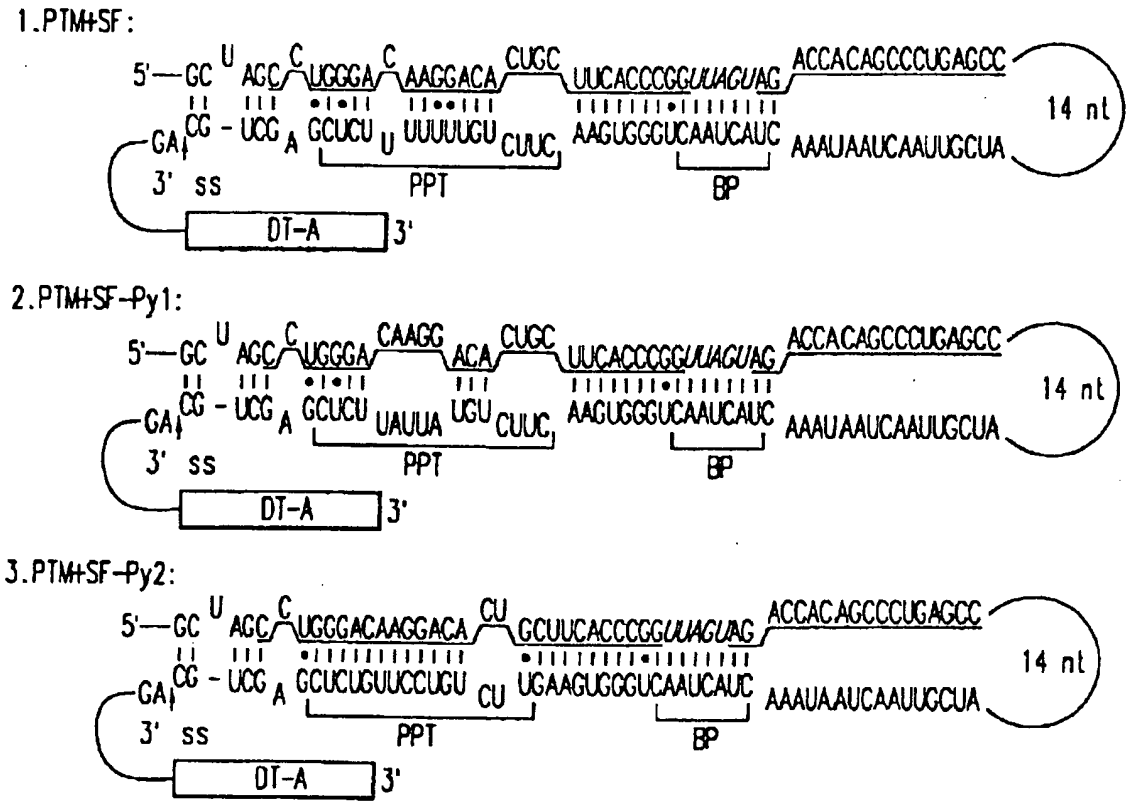

1.PTM+SF:
5'—GC U AGC C UGGGA C AAGGACA CUGC UUCACCCGGUUAGUAG ACCACAGCCCUGAGCC
CG - UCG A GCUCU U UUUUUGU CUUC AAGUGGGUCAAUCAUC AAAUAAUCAAUUGCUA
GA
3' ss
PPT
BP
14 nt
DT-A 3'
2.PTM+SF-Py1:
5'—GC U AGC C UGGGA CAAGG ACA CUGC UUCACCCGGUUAGUAG ACCACAGCCCUGAGCC
CG - UCG A GCUCU UAUUA UGU CUUC AAGUGGGUCAAUCAUC AAAUAAUCAAUUGCUA
GA
3' ss
PPT
BP
14 nt
DT-A 3'
3.PTM+SF-Py2:
5'—GC U AGC C UGGGACAAGGACA CU GCUUCACCCGGUUAGUAG ACCACAGCCCUGAGCC
CG - UCG A GCUCUGUUCCUGU CU UGAAGUGGGUCAAUCAUC AAAUAAUCAAUUGCUA
GA
3' ss
PPT
BP
14 nt
DT-A 3'

FIG.4A

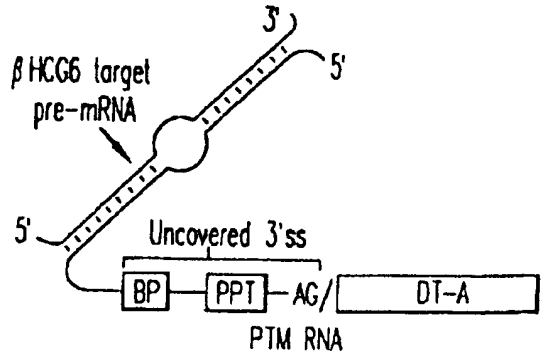

3'
5'
βHCG6 target
pre-mRNA
5'
Uncovered 3'ss
BP
PPT
AG/
DT-A
PTM RNA

FIG.4B

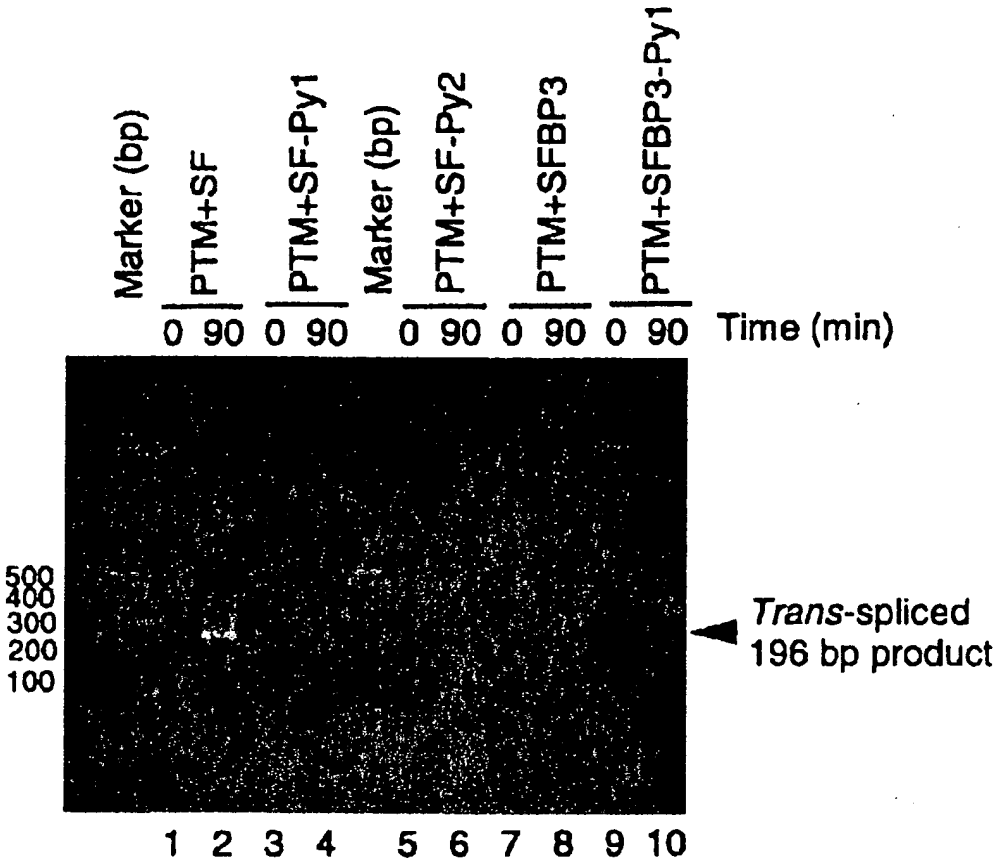

Marker (bp)
PTM+SF
PTM+SF-Py1
Marker (bp)
PTM+SF-Py2
PTM+SFBP3
PTM+SFBP3-Py1
0 90 0 90 0 90 0 90 0 90
Time (min)
500
400
300
200
100
Trans-spliced
196 bp product
1 2 3 4 5 6 7 8 9 10

FIG.4C

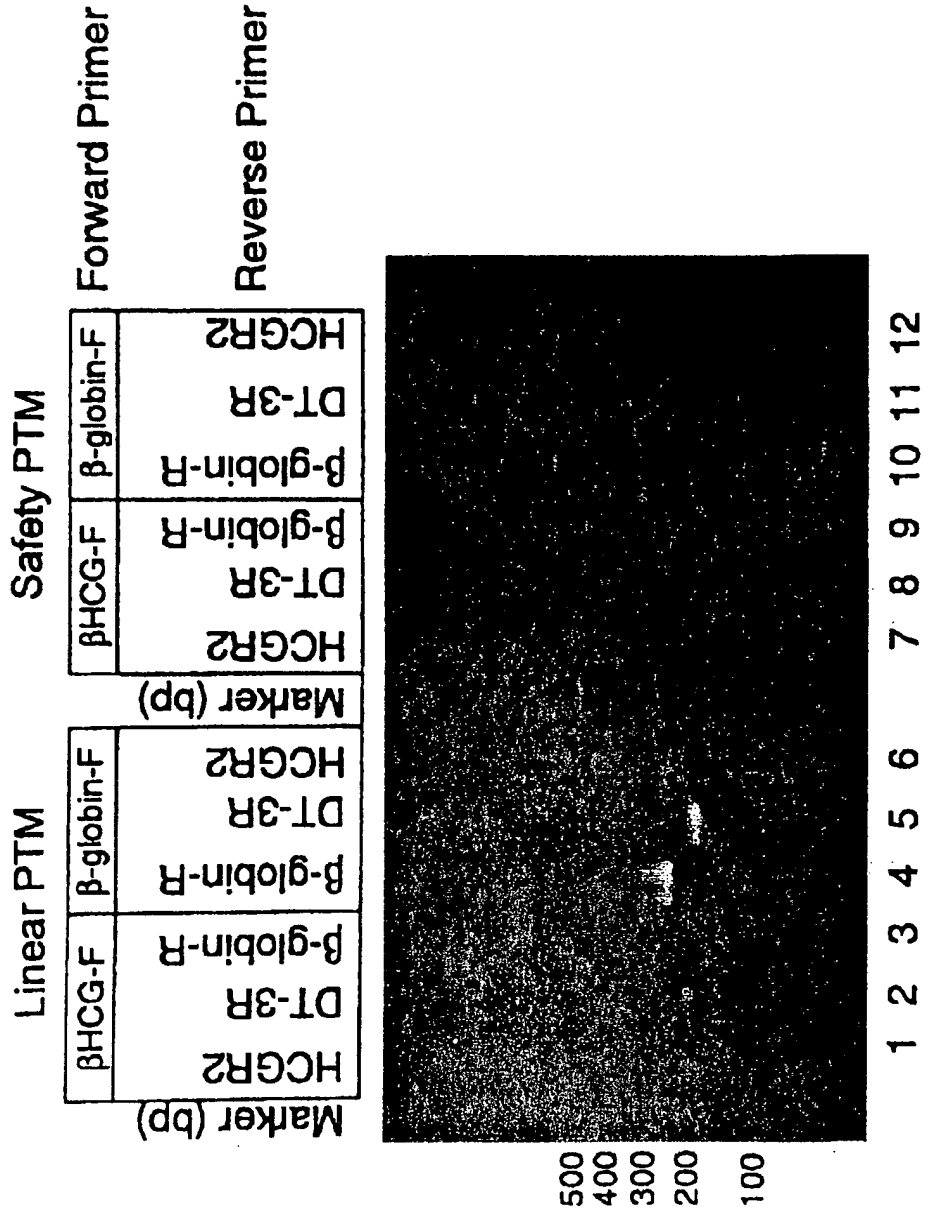

Linear PTM
Safety PTM
Forward Primer
Reverse Primer
βHCG-F
β-globin-F
Marker (bp)
HCGR2
DT-3R
β-globin-R
500
400
300
200
100
1 2 3 4 5 6 7 8 9 10 11 12

FIG.5

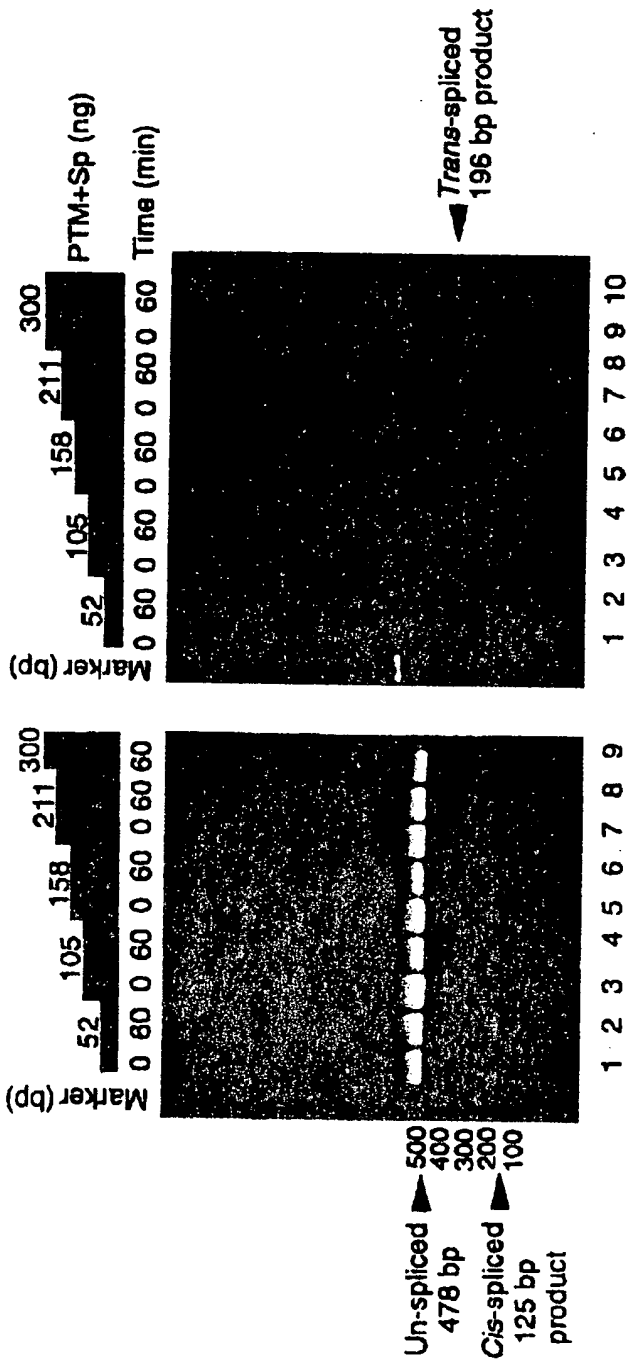

Marker (bp)
52
105
158
211
300
0 80 0 60 0 60 0 60 60
1 2 3 4 5 6 7 8 9
Un-spliced 478 bp
Cis-spliced 125 bp product
500
400
300
200
100
Marker (bp)
52
105
158
211
300
0 60 0 60 0 60 0 60 0 60 60
PTM+Sp (ng)
Time (min)
1 2 3 4 5 6 7 8 9 10
Trans-spliced 196 bp product

FIG.6A

FIG.6B

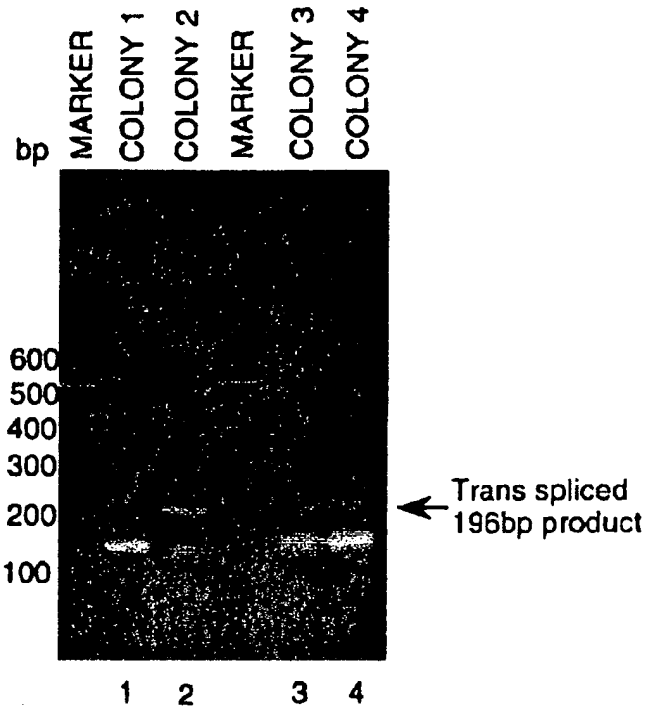

bp
MARKER
COLONY 1
COLONY 2
MARKER
COLONY 3
COLONY 4
600
500
400
300
200
100
Trans spliced
196bp product
1
2
3
4

FIG.7A

EXON 1 OF βHCG6

5'-CAGGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTGTT

1ST CODING NUCLEOTIDE OF DT-A

GATTCTTCTTAAATCTTTTGTGATGGAAAACTTTTCTTCGTACCACGGGACTA

AACCTGGTTATGTAGATTCCATTCAAAAA-3'

FIG.7B

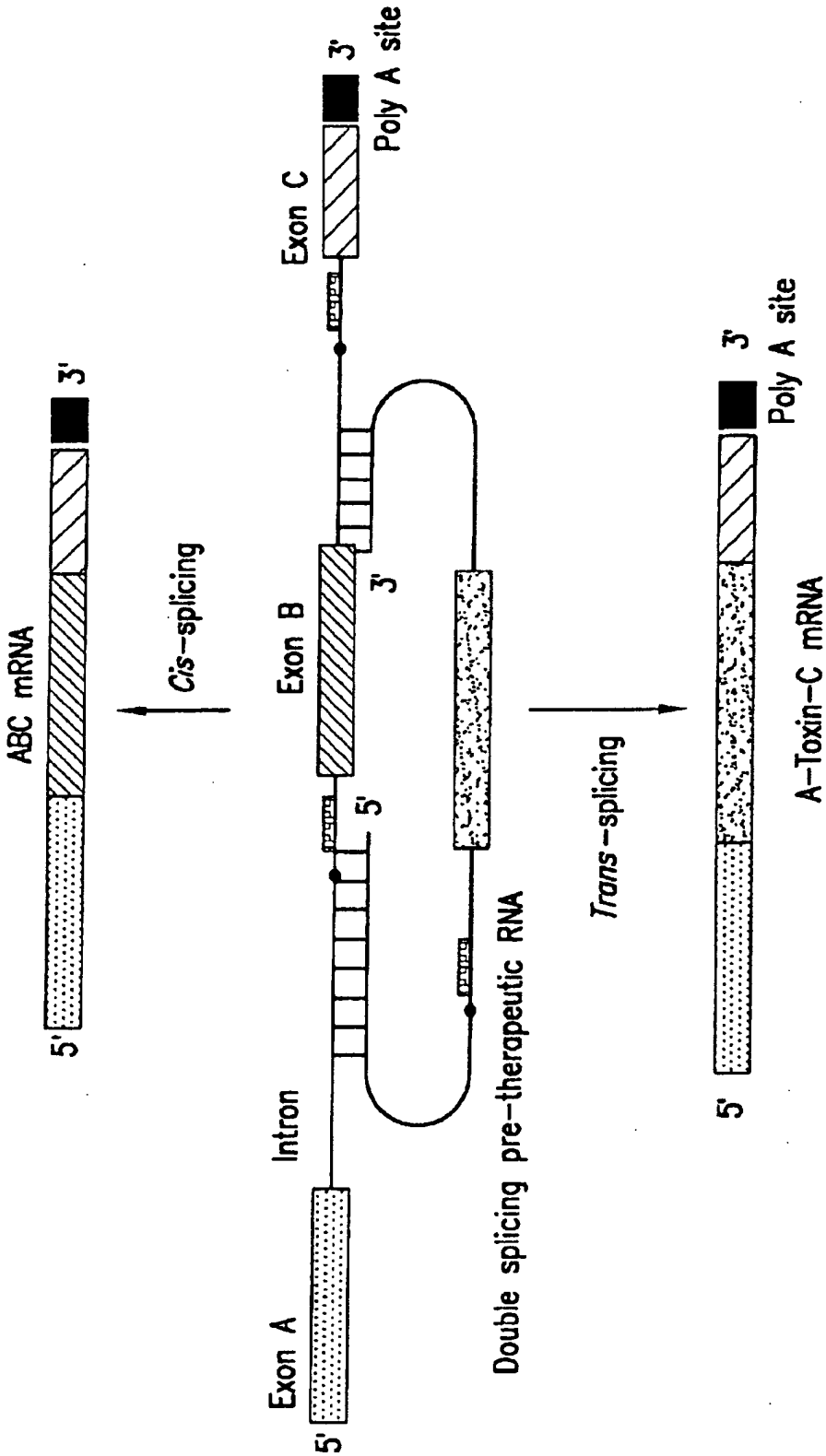
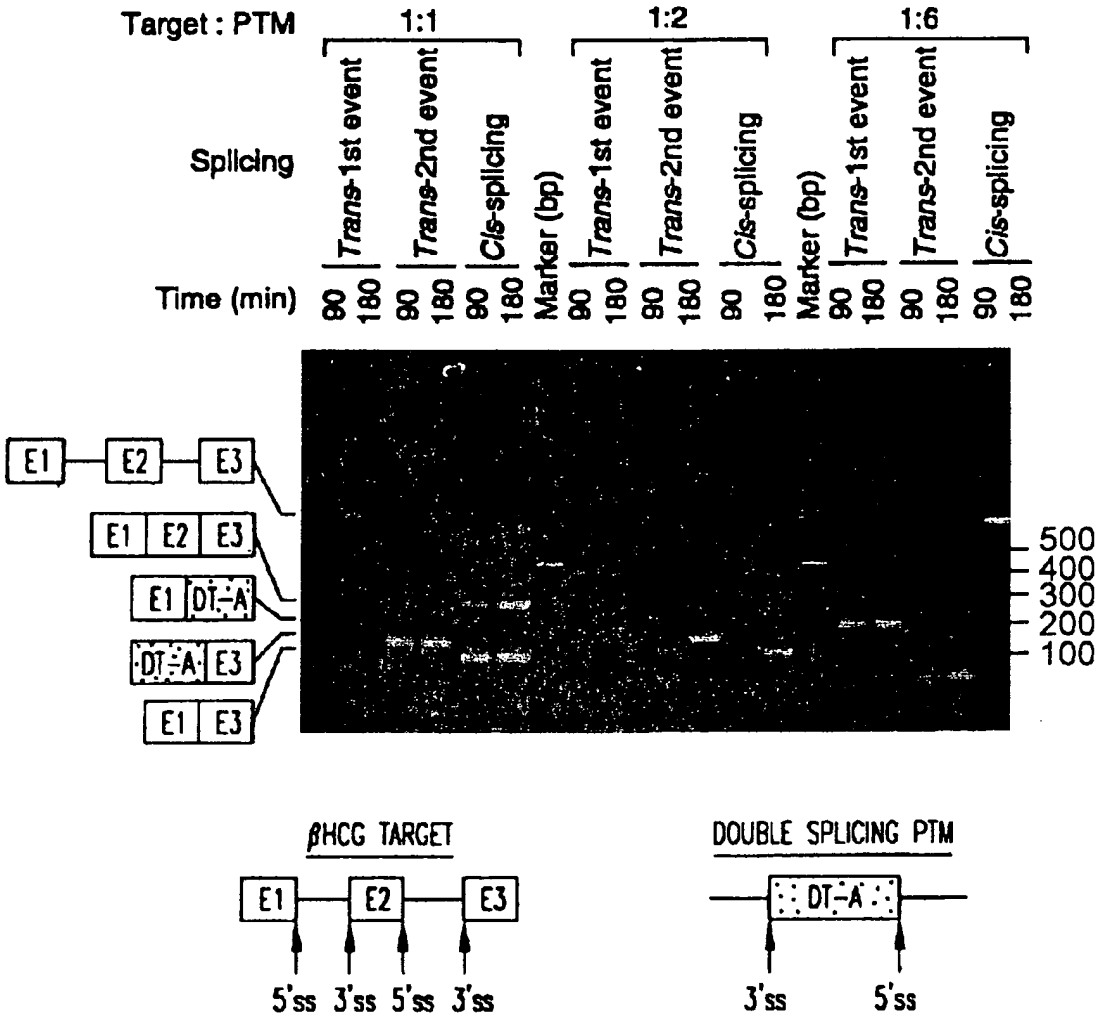

ABC mRNA
3'
5'
Cis-splicing
Exon A
Intron
Exon B
Exon C
3'
Poly A site
5'
3'
5'
Double splicing pre-therapeutic RNA
Trans-splicing
A-Toxin-C mRNA
3'
Poly A site
5'

FIG.8A



Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans- spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*- SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

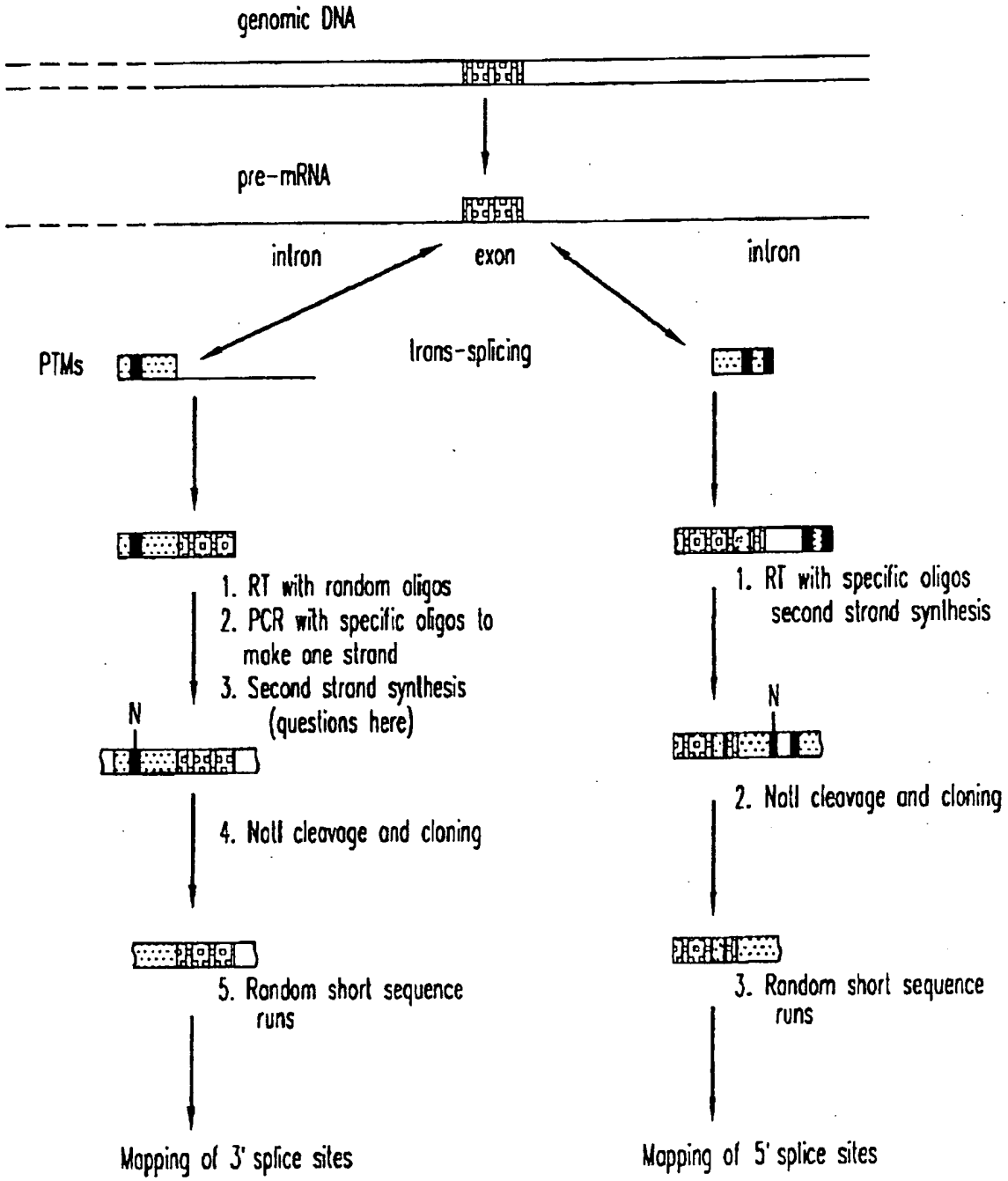

genomic DNA
pre-mRNA
intron
exon
intron
PTMs
trans-splicing
1. RT with random oligos
2. PCR with specific oligos to make one strand
3. Second strand synthesis (questions here)
N
4. NotI cleavage and cloning
5. Random short sequence runs
Mapping of 3' splice sites
1. RT with specific oligos second strand synthesis
N
2. NotI cleavage and cloning
3. Random short sequence runs
Mapping of 5' splice sites

FIG.9

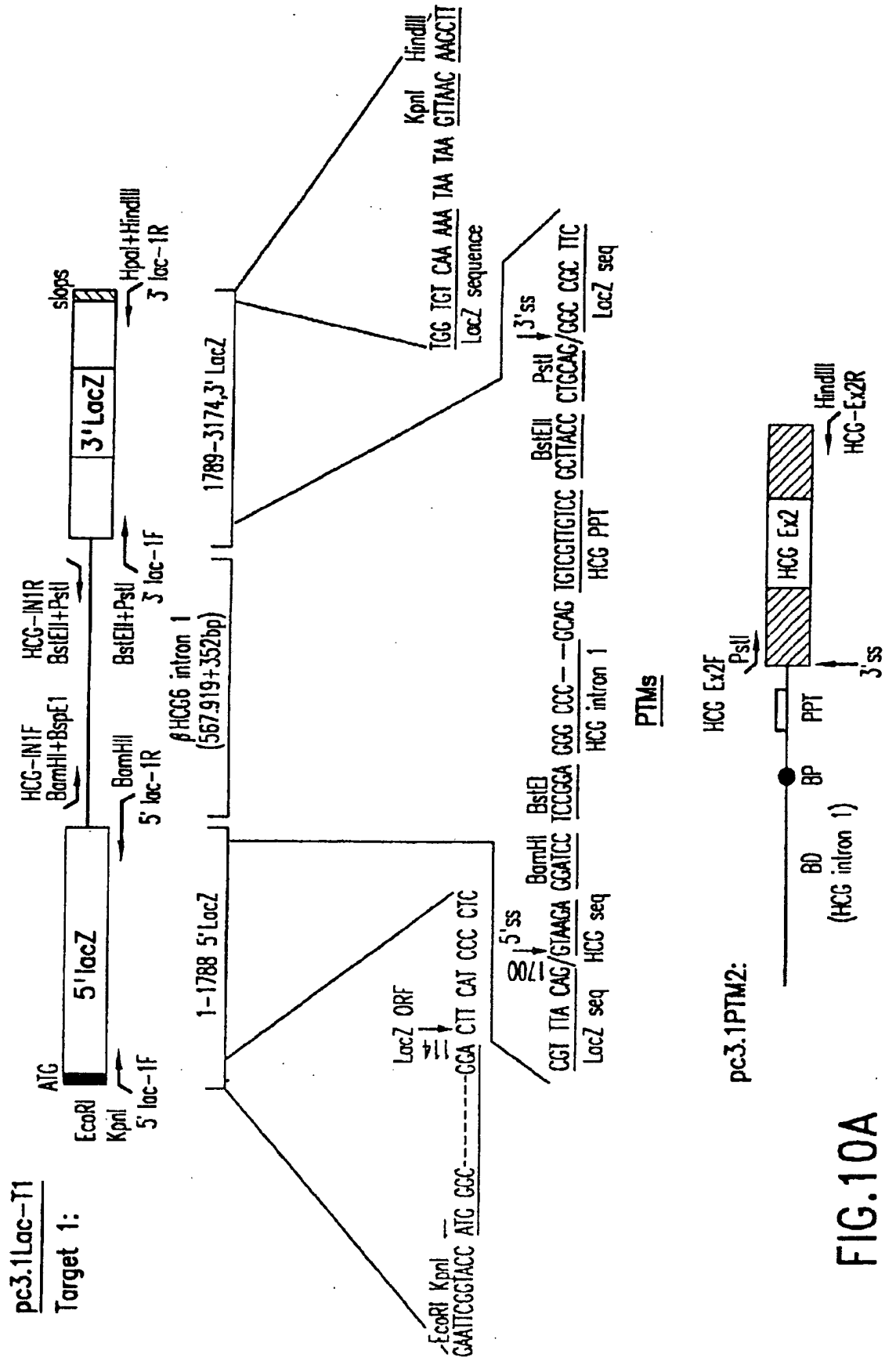

pc3.1Lac-T1
Target 1:
5'lacZ
3'LacZ
sIops
ATG
EcoRI
KpnI
5' lac-1F
HCG-IN1F
BamHI+BspE1
BamHI
5' lac-1R
HCG-IN1R
BstEII+PstI
BstEII+PstI
3' lac-1F
HpaI+HindIII
3' lac-1R
1-1788 5'LacZ
βHCG6 intron 1
(567.919+352bp)
1789-3174,3'LacZ
KpnI HindIII
GTTAAC AAGCTT
TGG TGT CAA AAA TAA TAA
LacZ sequence
BstEII PstI 3'ss
GCTTACC CTGCAG/GGC CGC TTC
LacZ seq
TGTCGTTGTCC
HCG PPT
LacZ ORF
EcoRI KpnI
GAATTCGGTACC ATG GGC-------CGA CTT CAT CCC CTC
5'ss
BamHI BstEI
CGT TTA CAG/GTAAGA GGATCC TCCGGA GGG CCC--CCAG
LacZ seq HCG seq
HCG intron 1
PTMs
pc3.1PTM2:
HCG Ex2F
PstI
HCG Ex2
HindIII
HCG-Ex2R
3'ss
PPT
BP
BD
(HCG intron 1)

FIG.10A

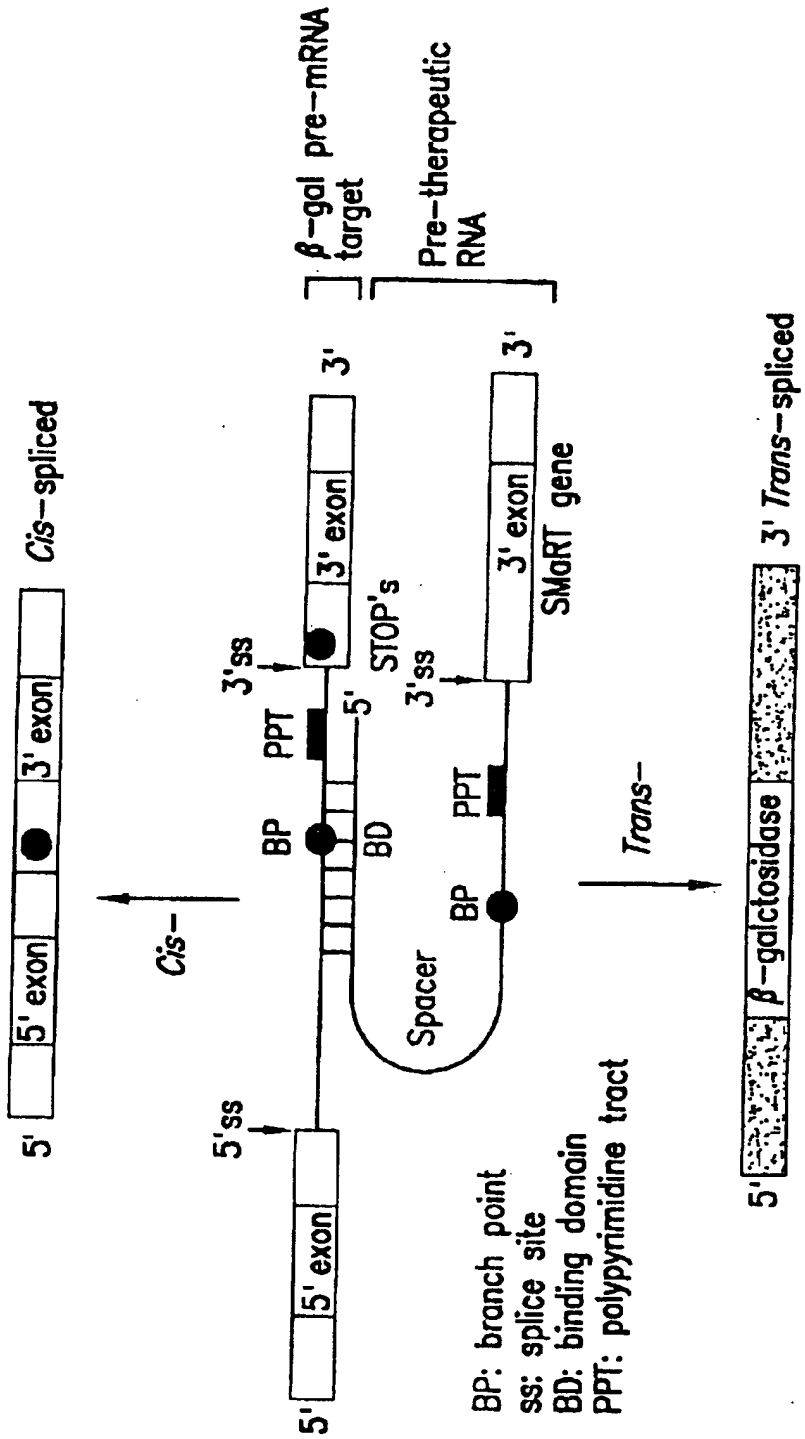

β-gal pre-mRNA target
Pre-therapeutic RNA
Cis-spliced
5' exon
3' exon
5'
3'
Cis-
3'ss
PPT
BP
BD
5'
STOP's
3' exon
3'
3'ss
PPT
BP
Spacer
SMaRT gene
Trans-
5'ss
5' exon
5'
5'
β-galctosidase
3' Trans-spliced
BP: branch point
ss: splice site
BD: binding domain
PPT: polypyrimidine tract

FIG.10B

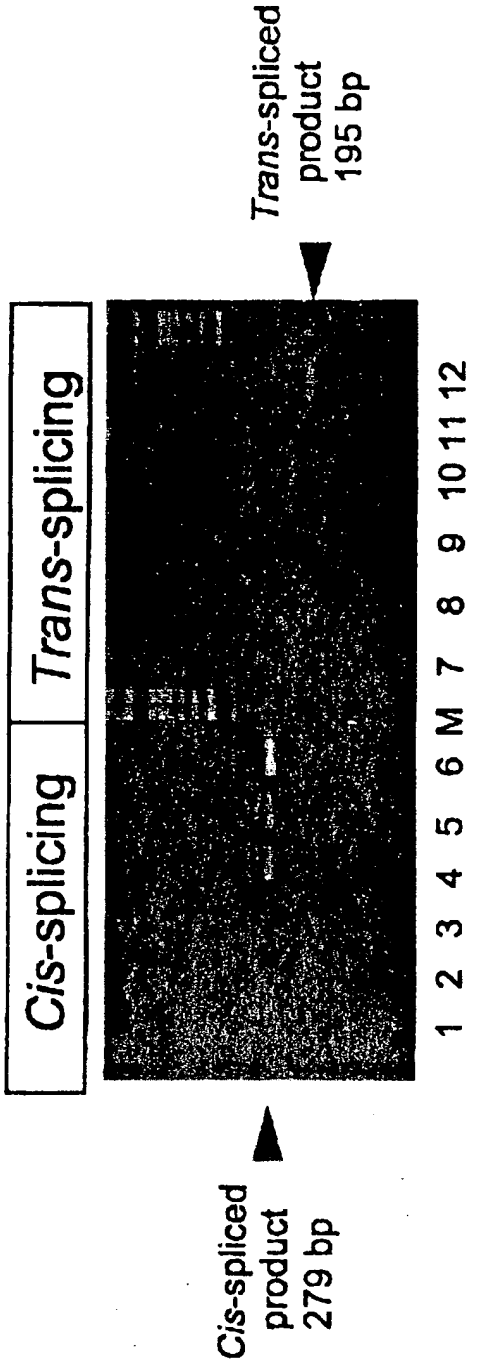

Trans-spliced product 195 bp
Cis-splicing
Trans-splicing
1 2 3 4 5 6 M 7 8 9 10 11 12
Cis-spliced product 279 bp

FIG.11A

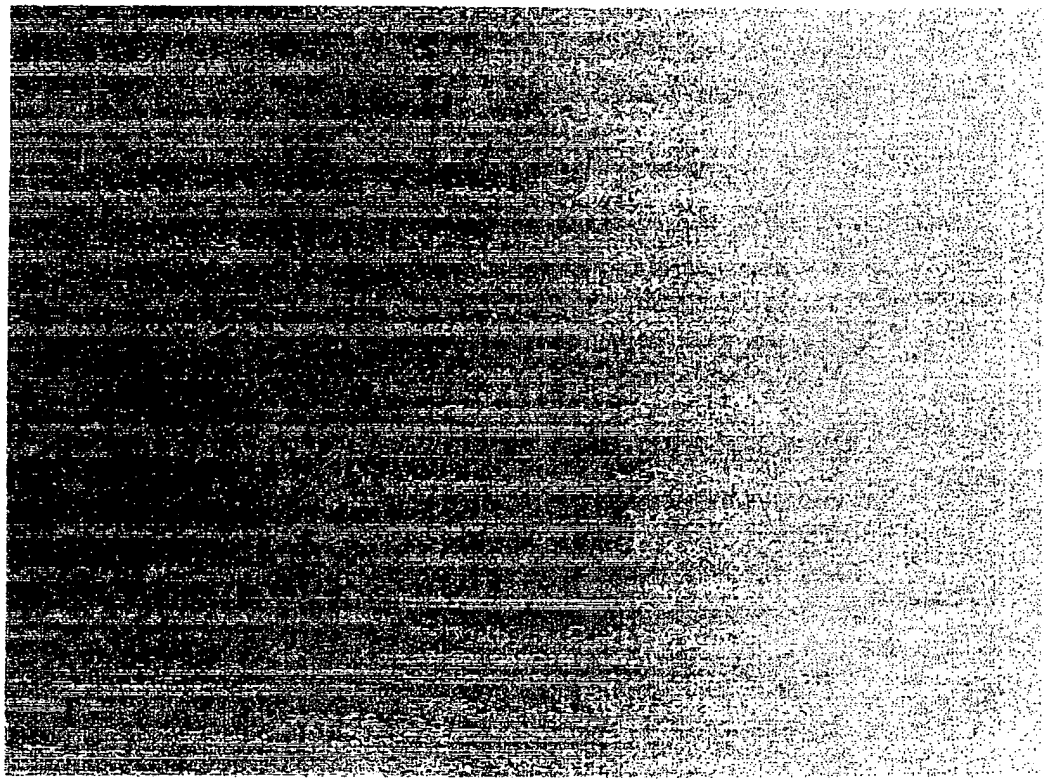


FIG.11B

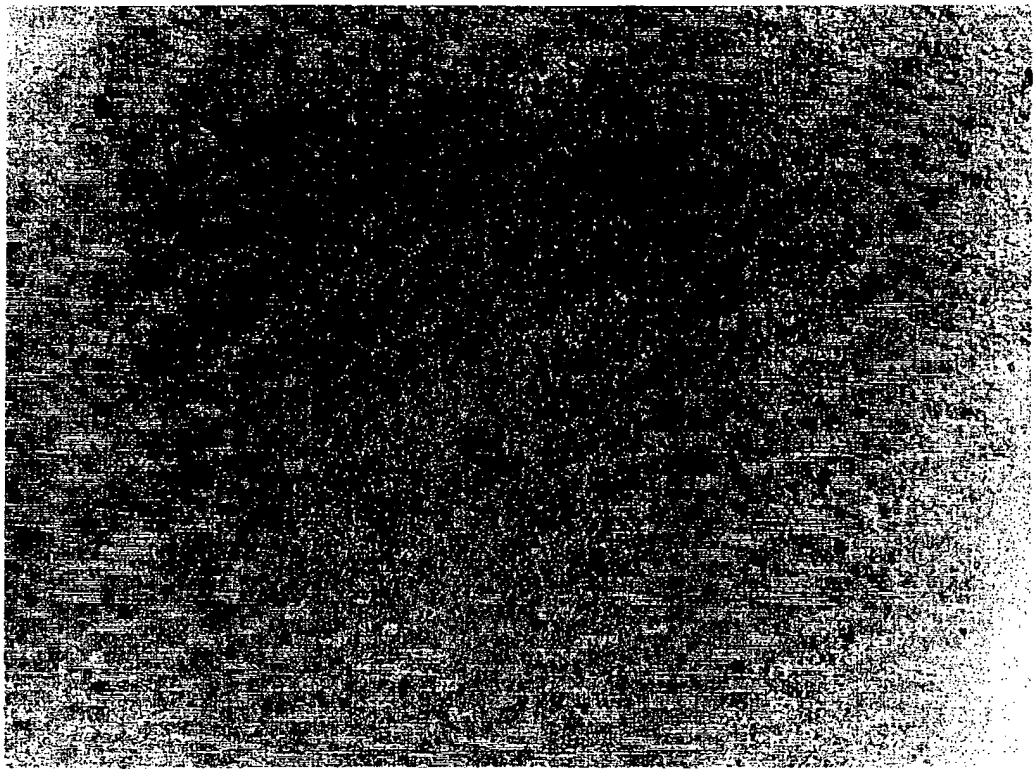


FIG.11C

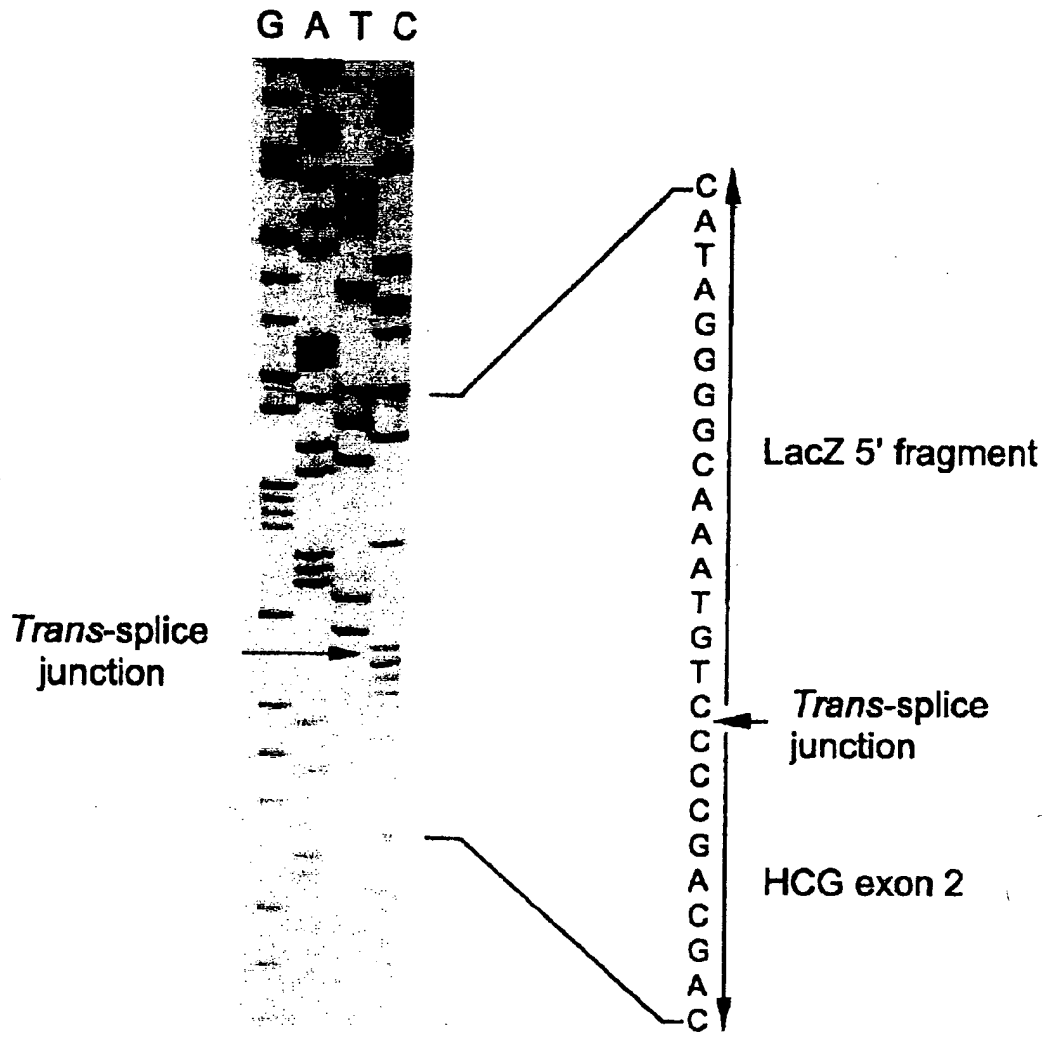

G A T C
C
A
T
A
G
G
G
G
C
A
A
A
A
T
G
T
C
C
C
C
C
G
A
C
G
A
C
LacZ 5' fragment
Trans-splice junction
Trans-splice junction
HCG exon 2

FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

BioLac-TR1

GGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTG

Splice junction

GCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGCGGCTTCGTCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTT

Lac-TR2

TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAG

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

BioLac-TR1

GGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

HCGR2

GAGCATGGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGGTGCCG

FIG.12B

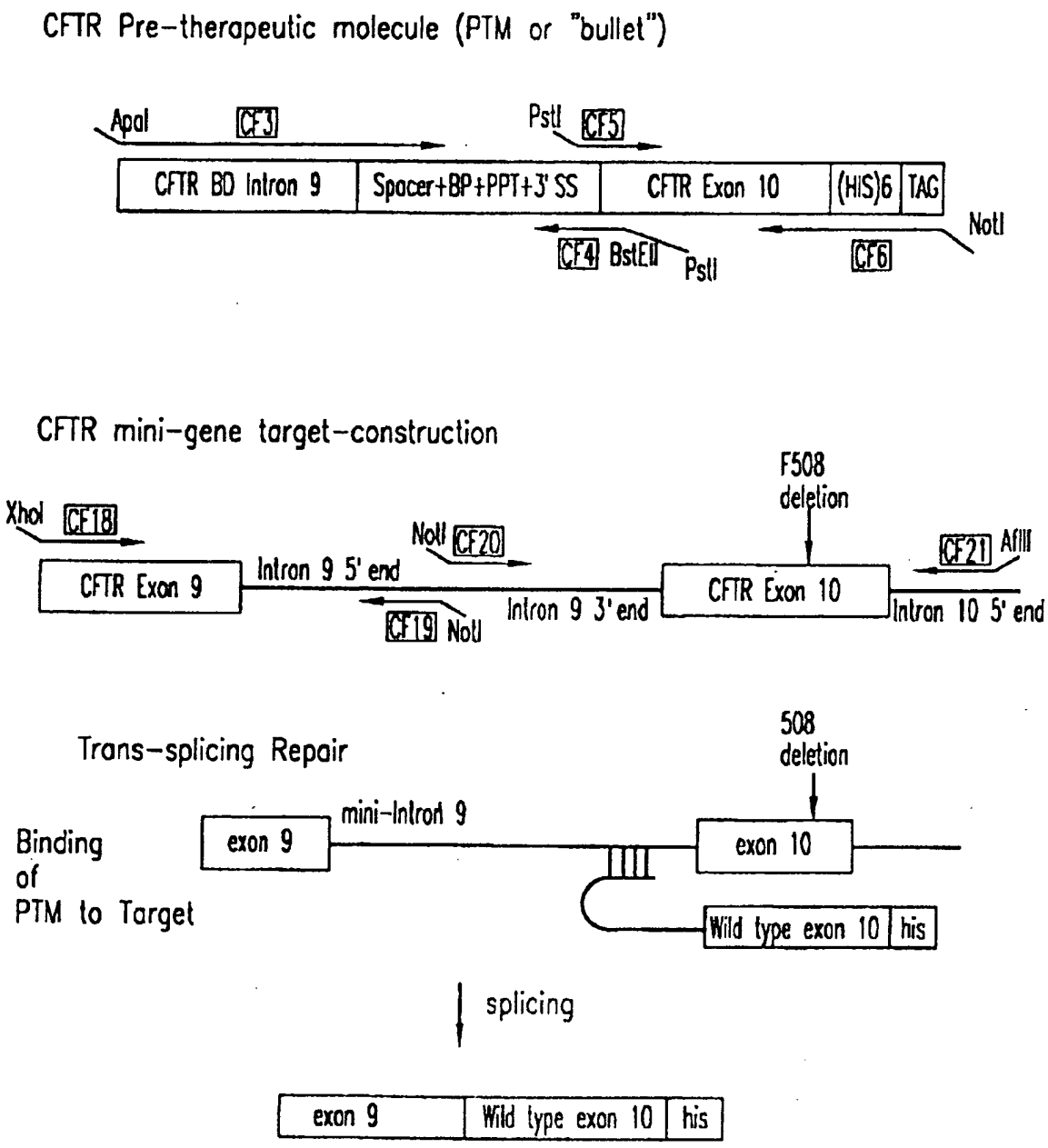

CFTR Pre-therapeutic molecule (PTM or "bullet")
ApaI
CF3
PstI
CF5
CFTR BD Intron 9
Spacer+BP+PPT+3' SS
CFTR Exon 10
(HIS)6
TAG
NotI
CF4
BstEII
PstI
CF6
CFTR mini-gene target-construction
F508 deletion
XhoI
CF18
NotI
CF20
CF21
AflII
CFTR Exon 9
Intron 9 5' end
Intron 9 3' end
CFTR Exon 10
Intron 10 5' end
CF19
NotI
Trans-splicing Repair
508 deletion
mini-Intron 9
Binding of PTM to Target
exon 9
exon 10
Wild type exon 10
his
splicing
exon 9
Wild type exon 10
his

FIG.13

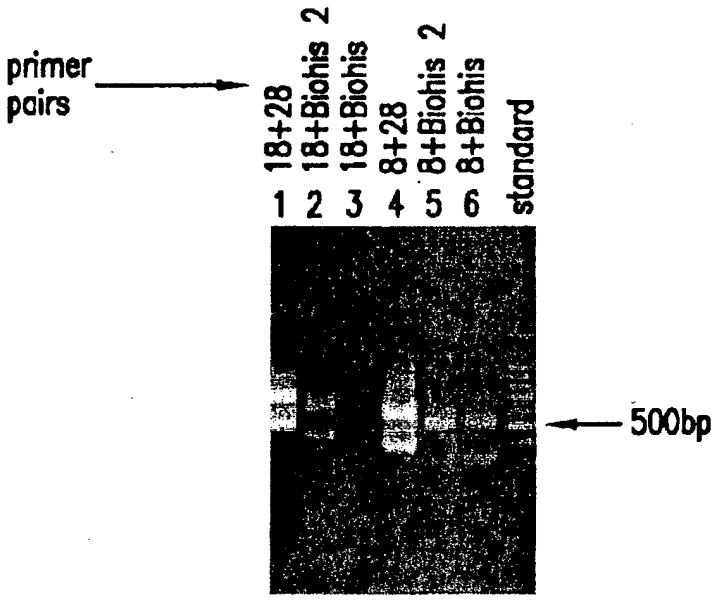

primer pairs
18+28
18+Biohis 2
18+Biohis
8+28
8+Biohis 2
8+Biohis
standard
1 2 3 4 5 6
500bp

FIG.14

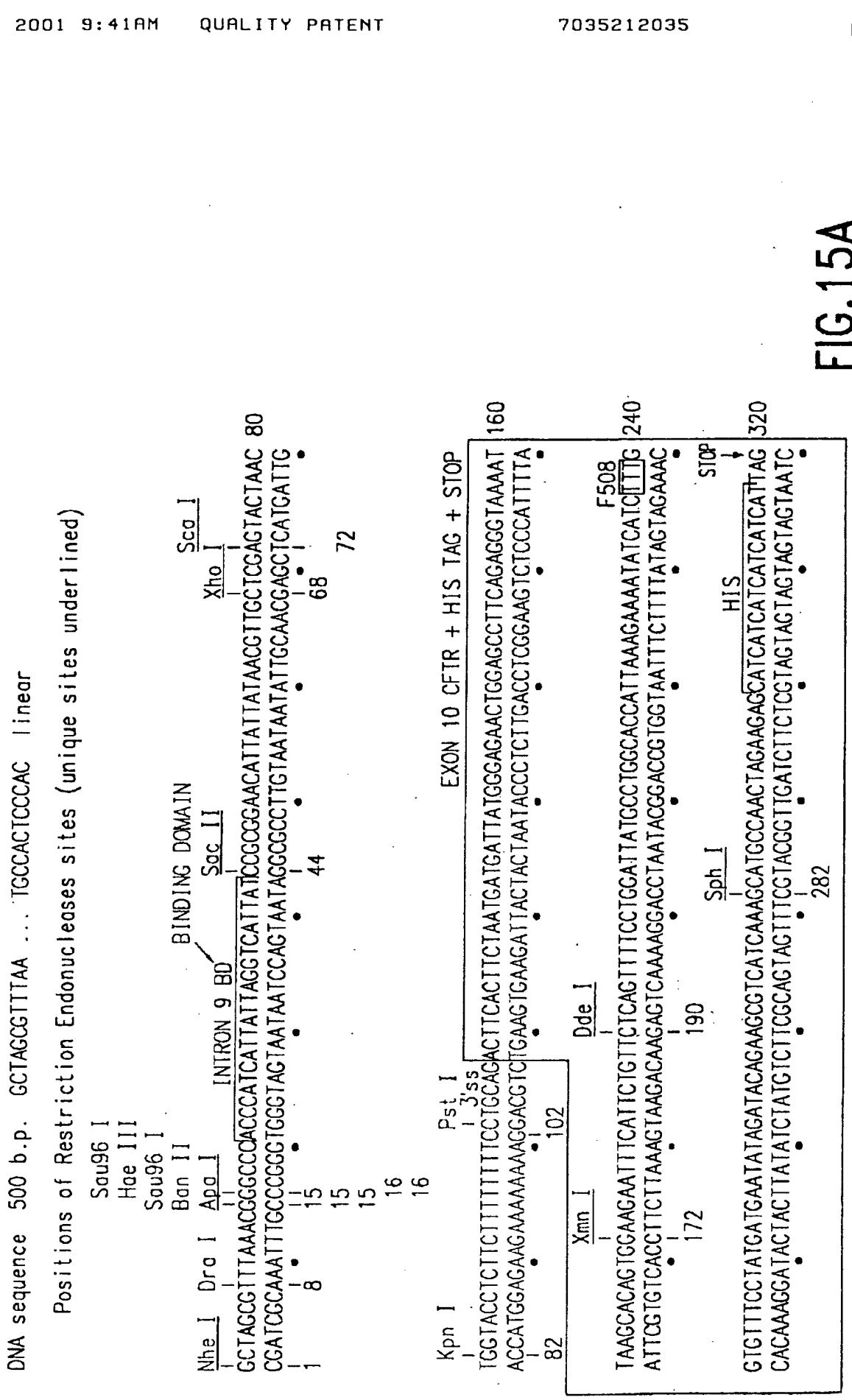
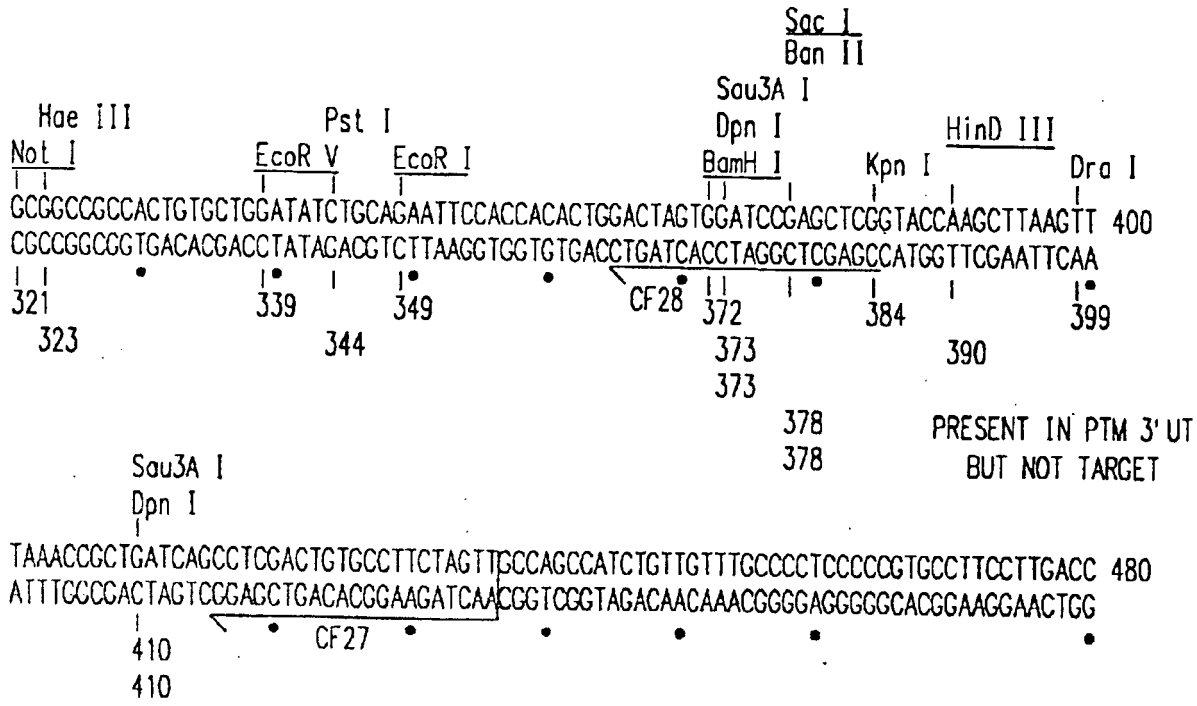

DNA sequence 500 b.p. GCTAGCGTTTAA .... TGCCACTCCCAC linear
Positions of Restriction Endonucleases sites (unique sites underlined)
Sau96 I
Hae III
Sau96 I
Ban II
BINDING DOMAIN
Sca I
Nhe I Dra I Apa I
INTRON 9 BD
Sac II
Xho I
GCTAGCGTTTAAACGGGCCCACCCATCATTATTAGGTCATTATCCGCGGAACATTATTATAACGTTGCTCGAGTACTAAC 80
CGATCGCAAATTTGCCCGGGTGGGTAGTAATAATCCAGTAATAGGCGCCTTGTAATAATATTGCAACGAGCTCATGATTG
1 8 15 44 68
15 72
15
16
16
Kpn I
Pst I
3'ss
EXON 10 CFTR + HIS TAG + STOP
TGGTACCTCTTCTTTTTTTTTCCTGCAGACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAAT 160
ACCATGGAGAAGAAAAAAAAGGACGTCTGAAGTGAAGATTACTACTAATACCCTCTTGACCTCGGAAGTCTCCCATTTTA
82 102
Xmn I
Dde I
F508
TAAGCACAGTGGAAGAATTTCATTCTGTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTG 240
ATTCGTGTCACCTTCTTAAAGTAAGACAAGAGTCAAAAGGACCTAATACGGACCGTGGTAATTTCTTTTATAGTAGAAAC
172 190
Sph I
HIS
STOP
GTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGCATCATCATCATCATTTAG 320
CACAAAGGATACTACTTATATCTATGTCTTCGCAGTAGTTTCGTACGGTTGATCTTCTCGTAGTAGTAGTAGTAATC
282

FIG.15A



Restriction Endonucleases site usage

Acc I	–	EcoR I	1	Nde I	–	Sau96 I	2
Apa I	1	EcoR V	1	Nhe I	1	Sca I	1
ApaL I	–	Hae II	–	Not I	1	Sma I	–
Avr II	–	Hae III	2	PflM I	–	Sph I	1
BamH I	1	HinC II	–	Pst I	2	Spl I	–
Ban II	2	HinD III	1	Pvu I	–	Ssp I	–
Bbe I	–	Hinf I	–	Pvu II	–	Stu I	–

FIG.15B

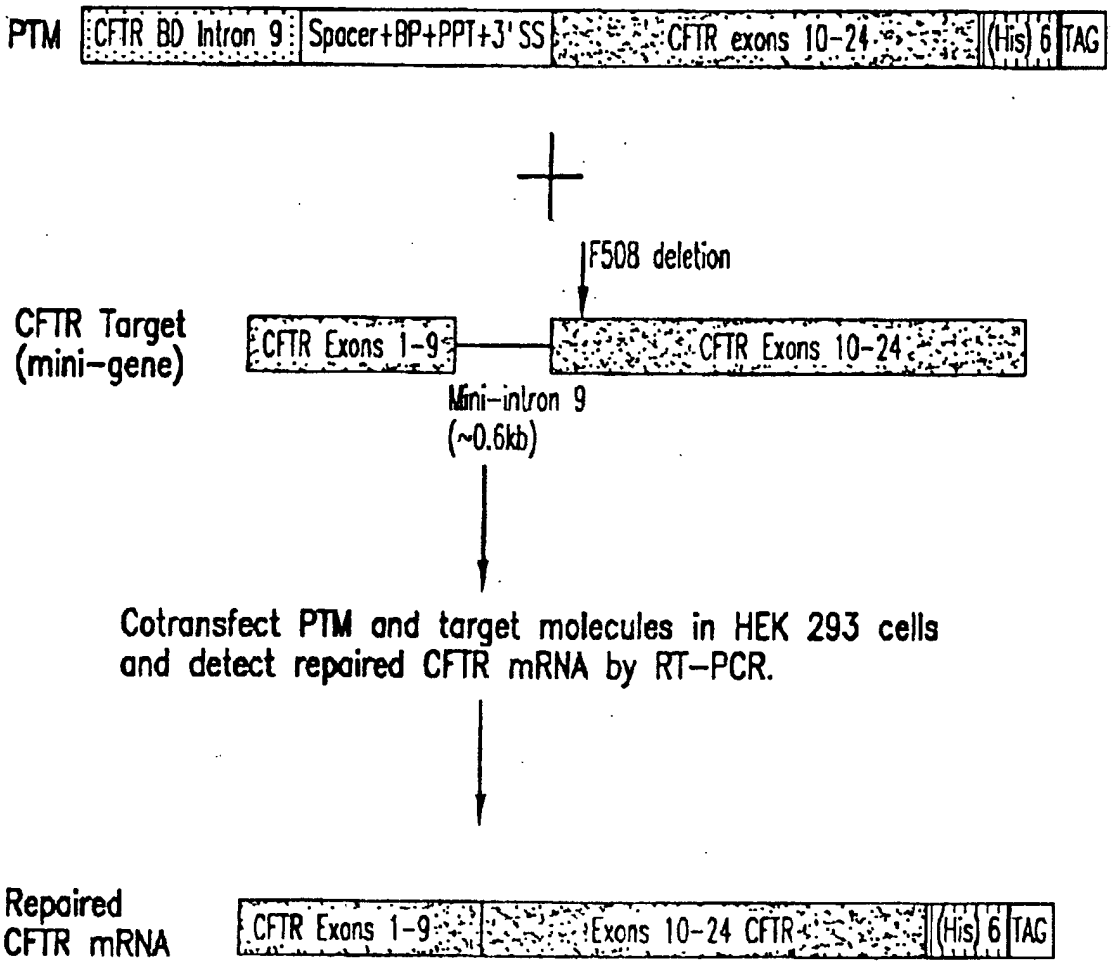

PTM
CFTR BD Intron 9
Spacer+BP+PPT+3' SS
CFTR exons 10-24
(His) 6
TAG
F508 deletion
CFTR Target
(mini-gene)
CFTR Exons 1-9
CFTR Exons 10-24
Mini-intron 9
(~0.6kb)
Cotransfect PTM and target molecules in HEK 293 cells
and detect repaired CFTR mRNA by RT-PCR.
Repaired
CFTR mRNA
CFTR Exons 1-9
Exons 10-24 CFTR
(His) 6
TAG

FIG.16

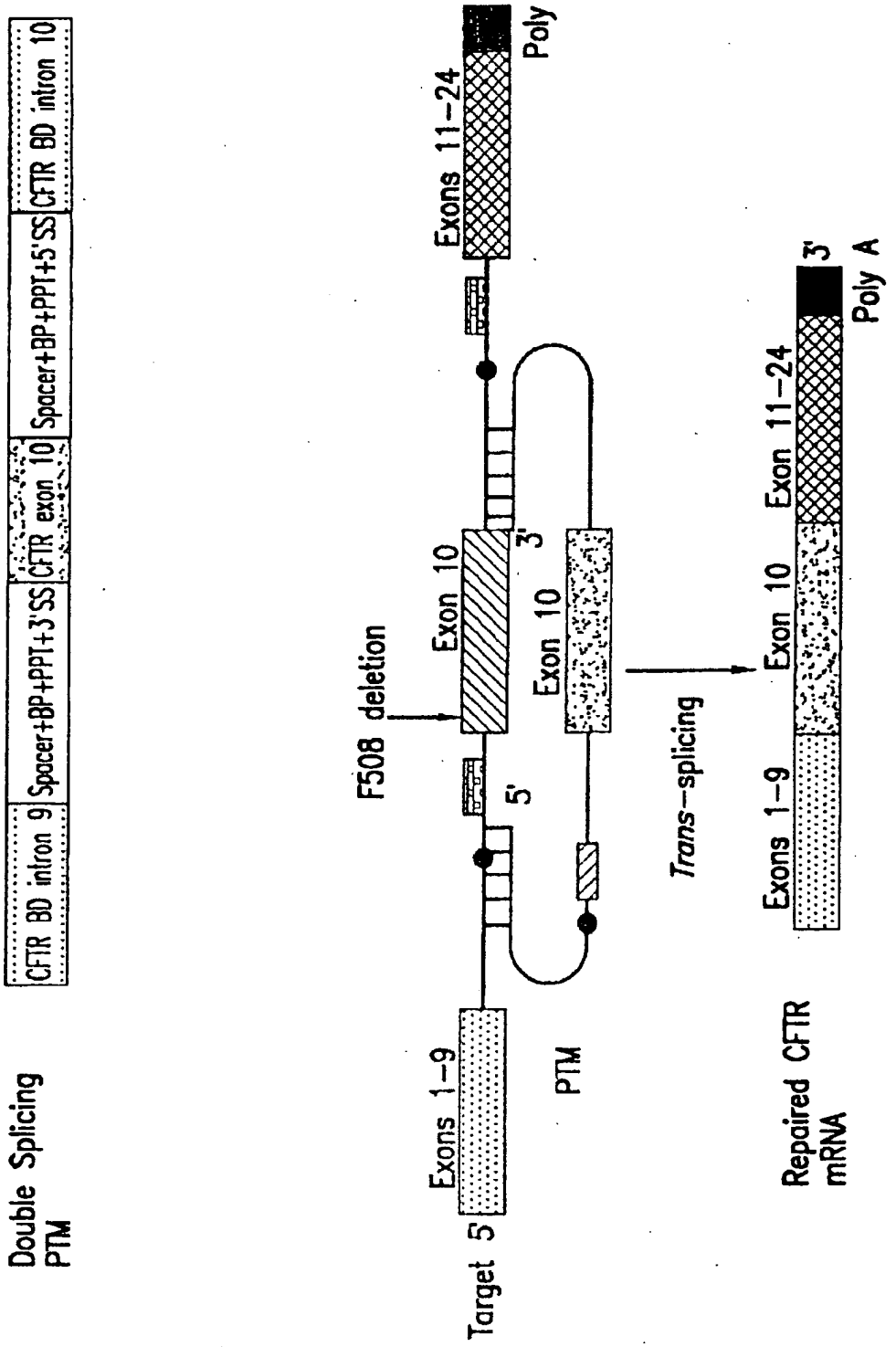

Double Splicing PTM
CFTR BD intron 9
Spacer+BP+PPT+3'SS
CFTR exon 10
Spacer+BP+PPT+5'SS
CFTR BD intron 10
Target 5'
Exons 1-9
PTM
F508 deletion
Exon 10
5'
Exon 10
3'
Exons 11-24
3'
Poly A
Trans-splicing
Repaired CFTR mRNA
Exons 1-9
Exon 10
Exon 11-24
3'
Poly A

FIG.17

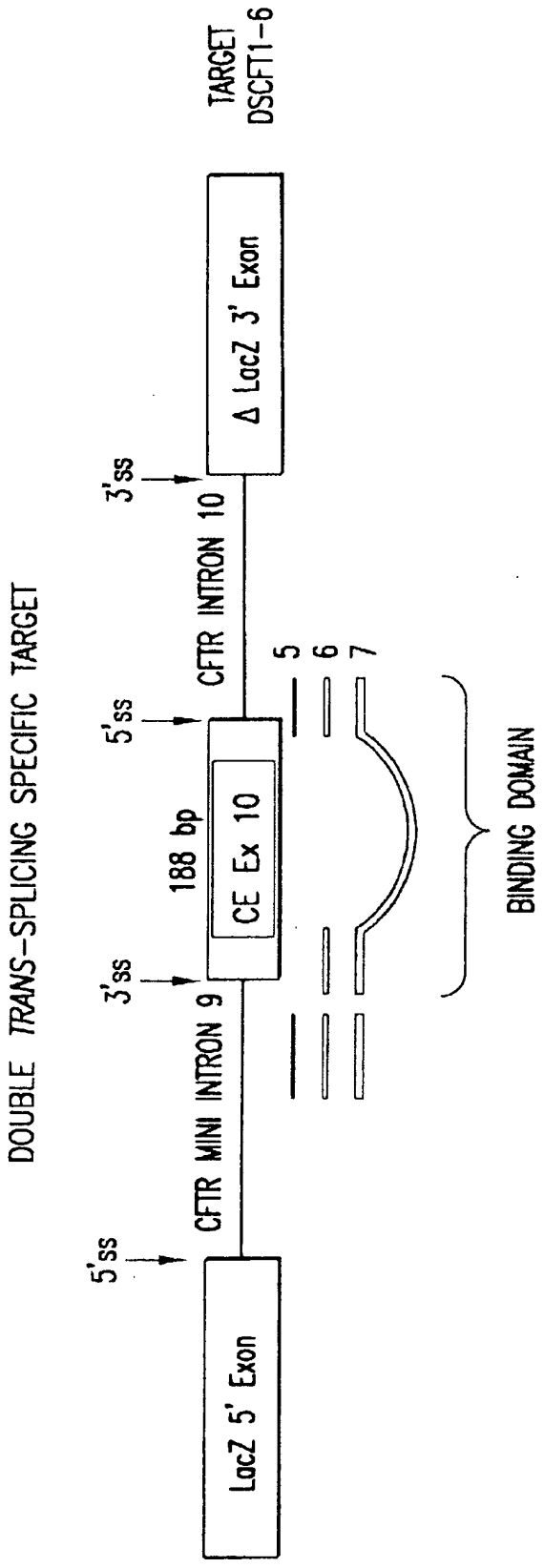

DOUBLE TRANS-SPLICING SPECIFIC TARGET
LacZ 5' Exon
5'ss
CFTR MINI INTRON 9
3'ss
188 bp
CE Ex 10
5'ss
CFTR INTRON 10
3'ss
Δ LacZ 3' Exon
TARGET
DSCFT1-6
5
6
7
BINDING DOMAIN

FIG.18

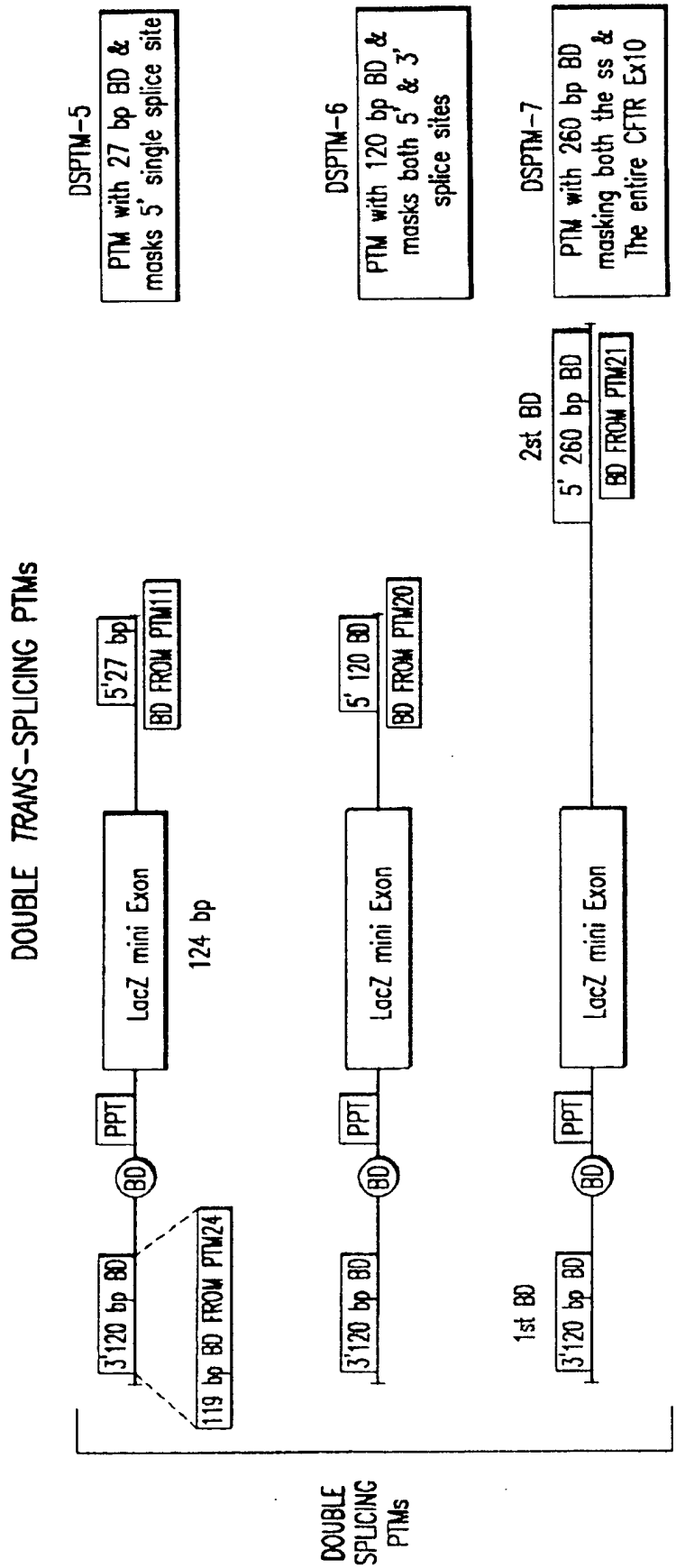

DOUBLE TRANS-SPLICING PTMs
DSPTM-5
PTM with 27 bp BD & masks 5' single splice site
DSPTM-6
PTM with 120 bp BD & masks both 5' & 3' splice sites
DSPTM-7
PTM with 260 bp BD masking both the ss & The entire CFTR Ex10
2st BD
5' 260 bp BD
BD FROM PTM21
5'27 bp
BD FROM PTM11
5' 120 BD
BD FROM PTM20
LacZ mini Exon
124 bp
PPT
BD
3'120 bp BD
119 bp BD FROM PTM24
1st BD
DOUBLE SPLICING PTMs

FIG.19

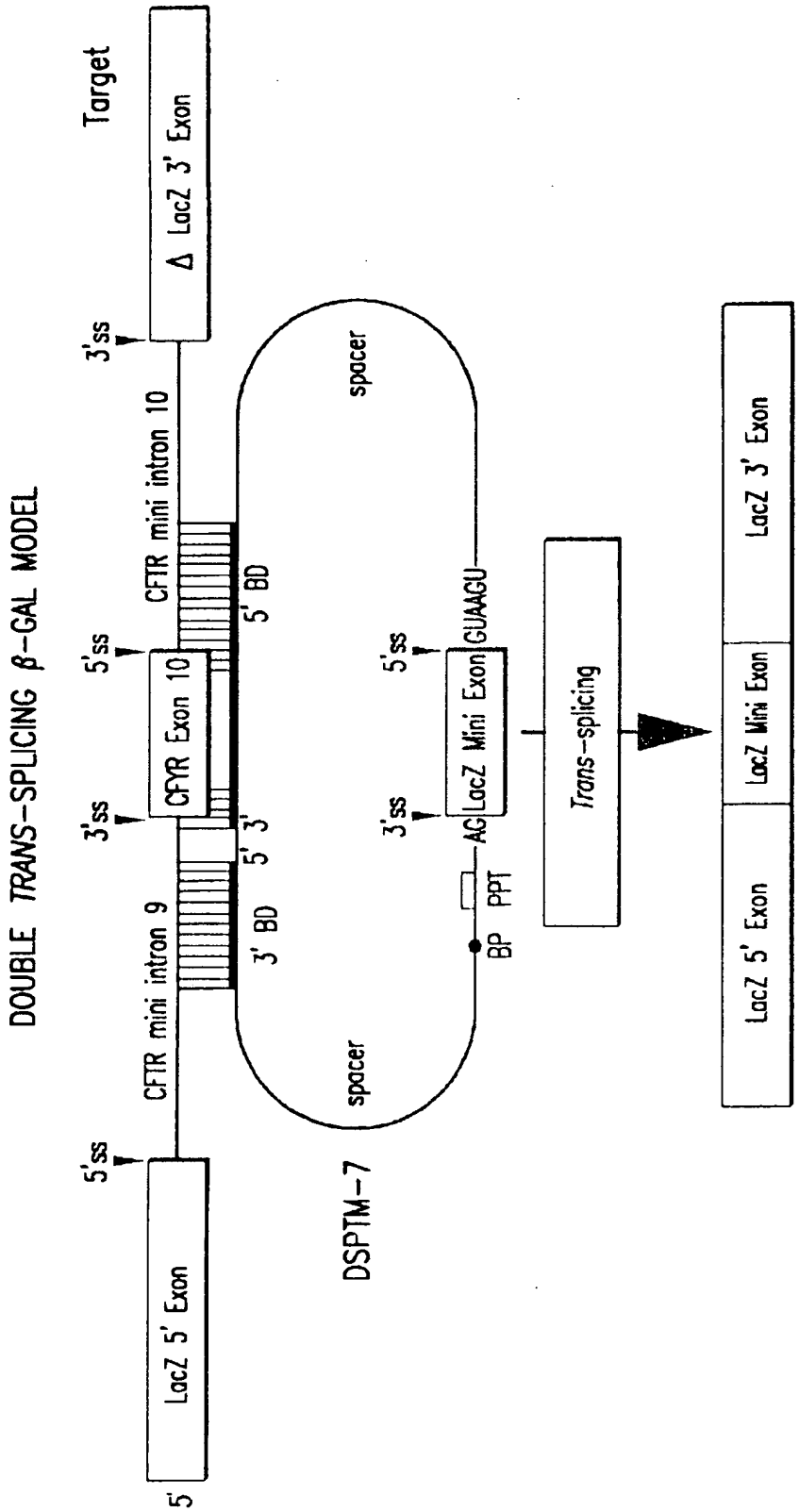
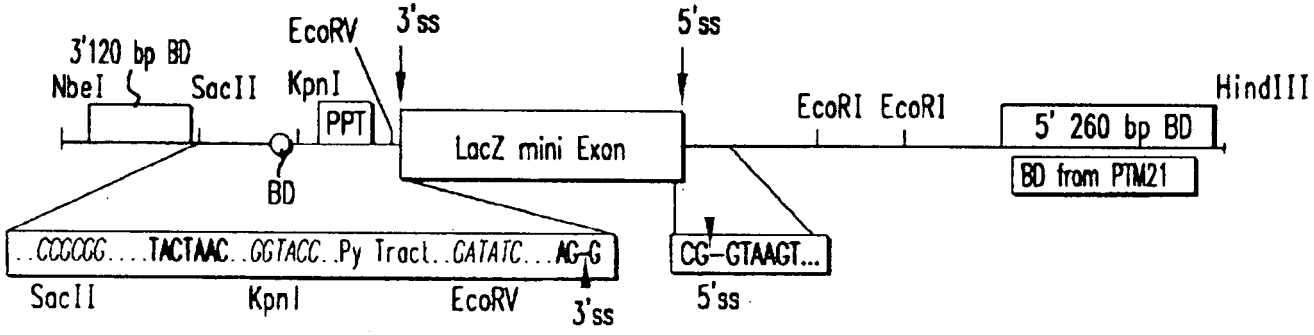

DOUBLE TRANS-SPLICING β-GAL MODEL
Target
Δ LacZ 3' Exon
3'ss
CFTR mini intron 10
5'ss
CFYR Exon 10
3'ss
CFTR mini intron 9
5'ss
LacZ 5' Exon
5'
5' BD
3' BD
5' 3'
spacer
spacer
DSPTM-7
5'ss
LacZ Mini Exon
GUAAGU
3'ss
AG
BP
PPT
Trans-splicing
LacZ 5' Exon
LacZ Mini Exon
LacZ 3' Exon
Repaired LacZ mRNA

FIG.20



(1) 3' BD (120 BP): CATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATTTGTAAAGATTCTATTAACTCATTTGATTC
AAAATATTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site: BP Kpn I PPT EcoRV 3'ss LacZ mini exon
TACTAAC T *GGTACC* TCTTCTTTTTTTTTT *GATATC* CTGCAG GGC GGC

(4) 5' donor site and 2nd spacer sequence: LacZ mini exon 5'ss
TGA ACG *GTAAGT* GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACG
CTAAGATCCACCGG

(5) 5' BD (260 BP): TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTT*GAATTC*ATGCTTTGATGACGCTTCTGTATCTATATTCATCATTGGAA
ACACCAATGATTTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA
AAAAACCCTCT*GAATTC*TCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTATTAACTCA
TTATCAAATCACGC

FIG.21

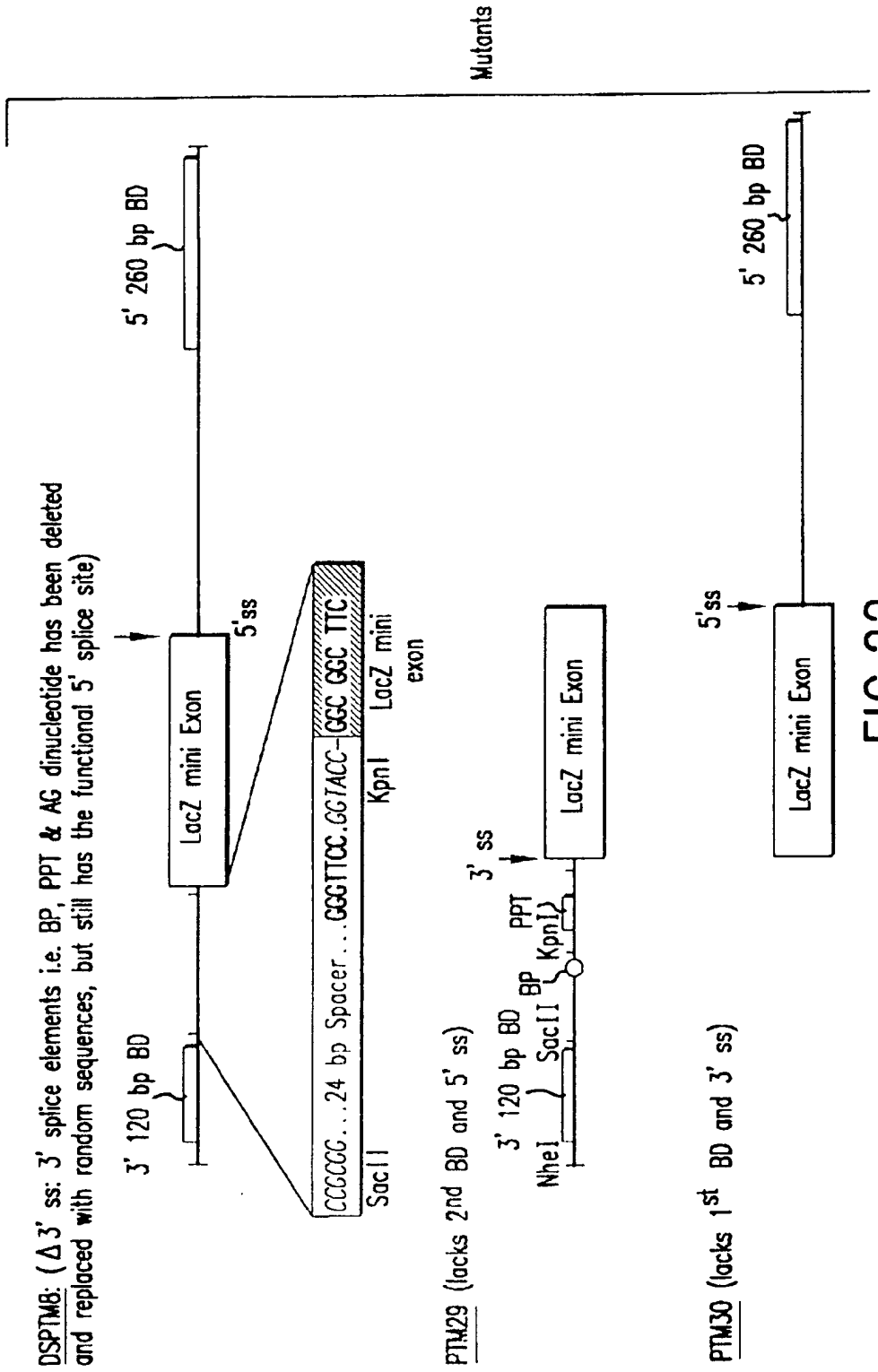

Mutants
DSPTM8: (Δ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)
5' 260 bp BD
3' 120 bp BD
LacZ mini Exon
5'ss
CCCCCC...24 bp Spacer...GGGTTCC.GGTACC-GGC GGC TTC
SacII
KpnI
LacZ mini exon
PTM29 (lacks 2nd BD and 5' ss)
3' ss
3' 120 bp BD
PPT
NheI
SacII
BP
KpnI
LacZ mini Exon
PTM30 (lacks 1st BD and 3' ss)
5' 260 bp BD
5'ss
LacZ mini Exon

FIG.22

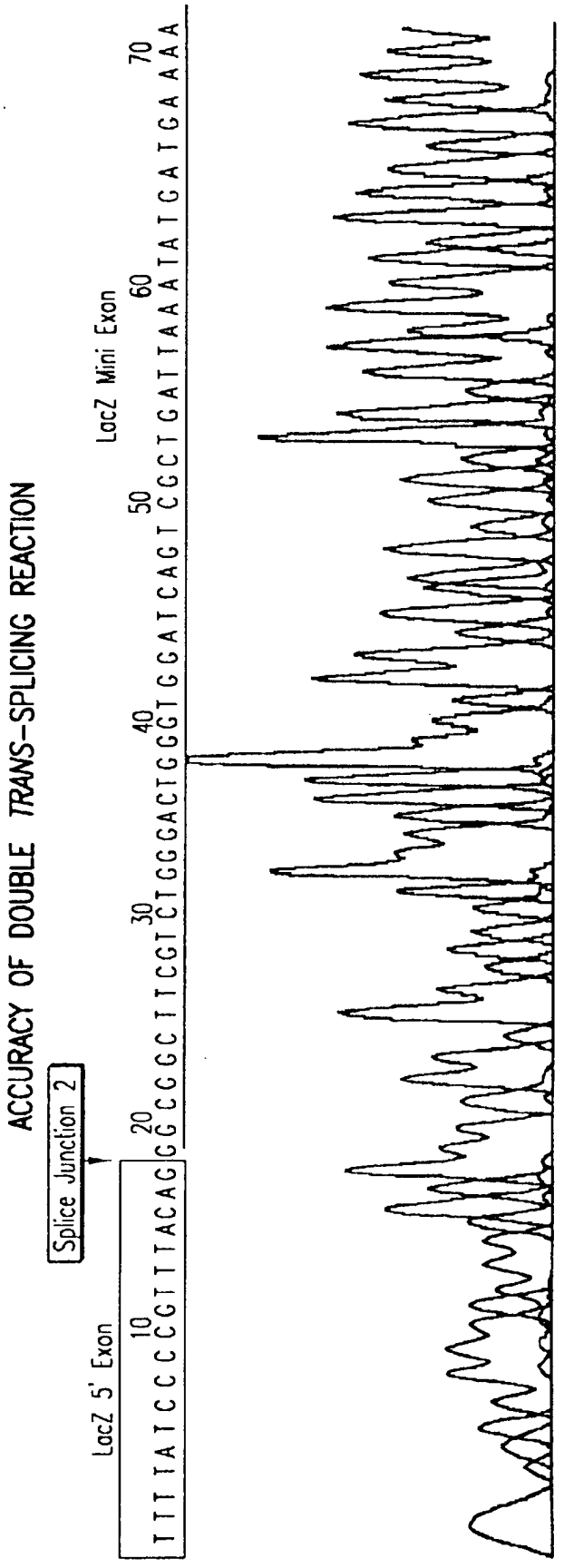

ACCURACY OF DOUBLE TRANS-SPLICING REACTION
LacZ 5' Exon
Splice Junction 2
LacZ Mini Exon
10
20
30
40
50
60
70
T T T A T C C C C G T T T A C A G G C G C G G C T T C G T C T G G A C T G G T G G A T C A G T C G C T G A T T A A A T A T G A T G A A A A

FIG.23A

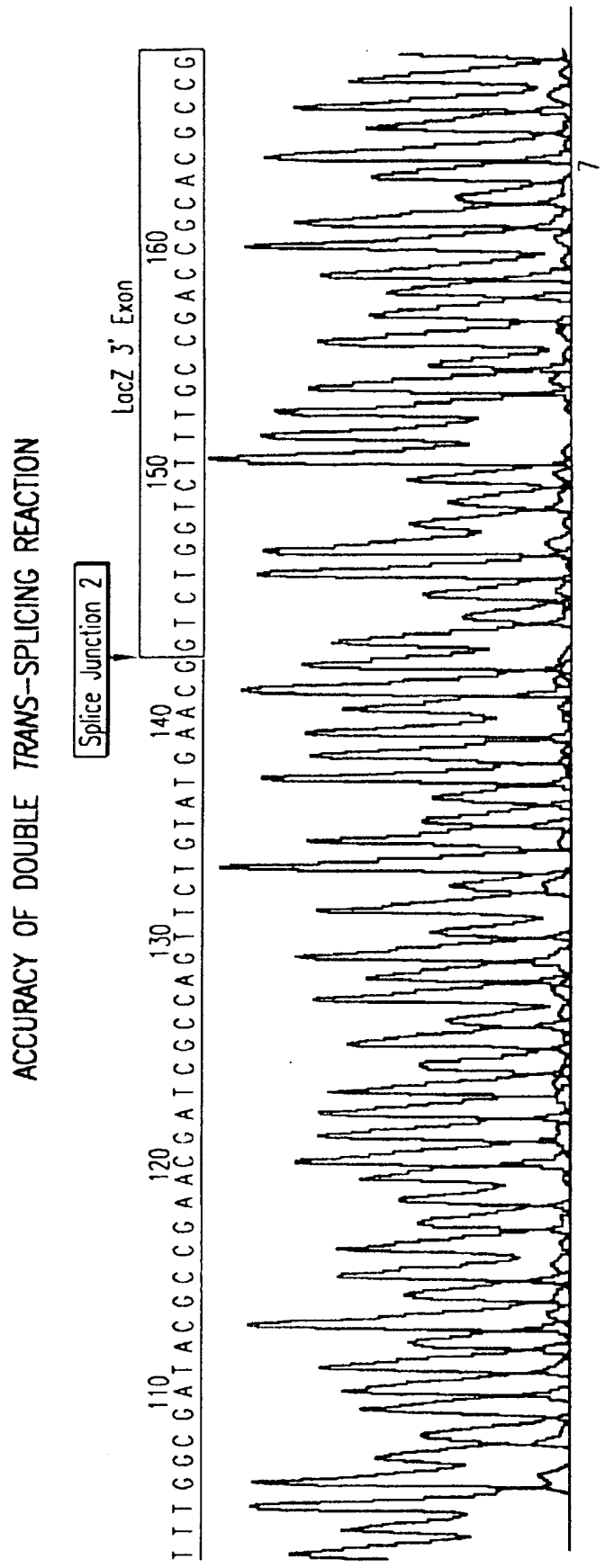

ACCURACY OF DOUBLE TRANS-SPLICING REACTION
Splice Junction 2
LacZ 3' Exon
110
120
130
140
150
160
T T T G C C G A T A C G C C G A A C G A T C G C C A G T T C T G T A T G A A C G G T C T T T G C C G A C C G C A C G C C G
7

FIG.23B

Double *Trans*-splicing Produces Full-length Protein

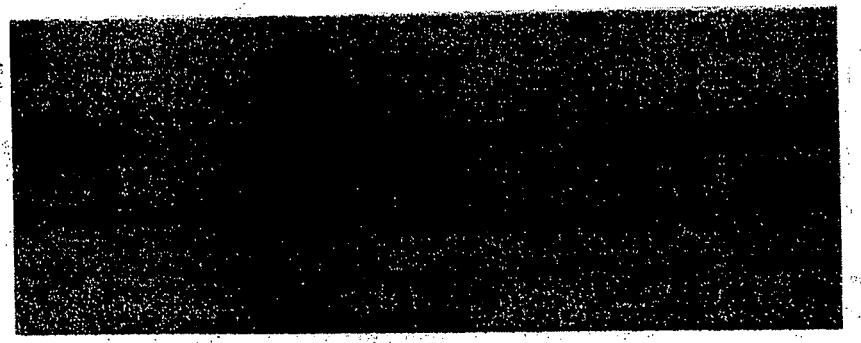


Figure 24

Lane 1: DSCFT1.6 Target alone	25 µg
Lane 2: DSPTM7	25 µg
Lane 3 Target + PTM #6	25 µg
Lane 4: Target + PTM #9	25 µg
Lane 5: Delta 3' splice mutant alone	25 µg
Lane 6: Target + Delta 3' ss	25 µg
Lane 7: Target+PTM29+30 (mutants)	25 µg

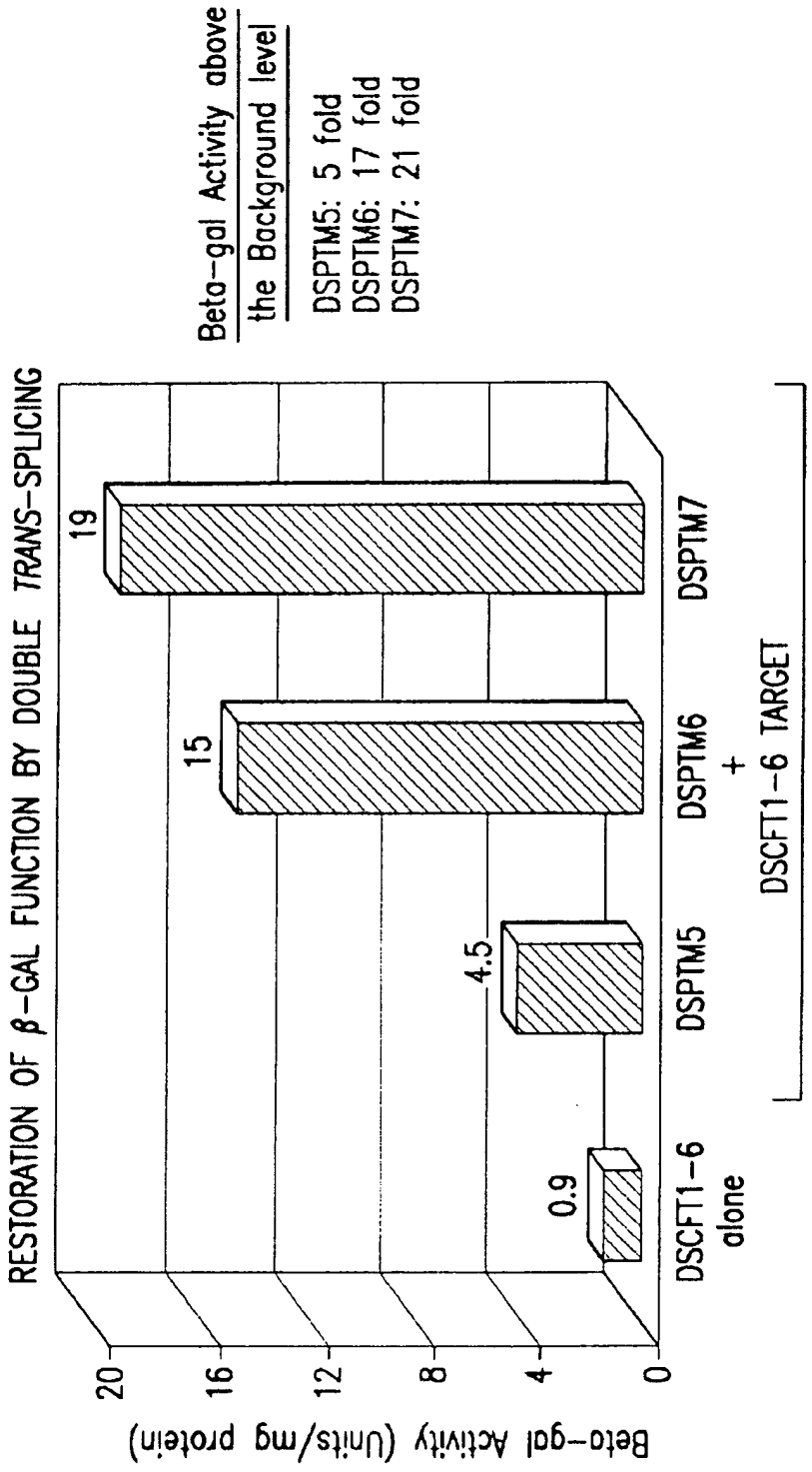

RESTORATION OF β-GAL FUNCTION BY DOUBLE TRANS-SPLICING
Beta-gal Activity above the Background level
DSPTM5: 5 fold
DSPTM6: 17 fold
DSPTM7: 21 fold
Beta-gal Activity (Units/mg protein)
0
4
8
12
16
20
0.9
4.5
15
19
DSCFT1-6 alone
DSPTM5
DSPTM6
DSPTM7
+
DSCFT1-6 TARGET

FIG.25

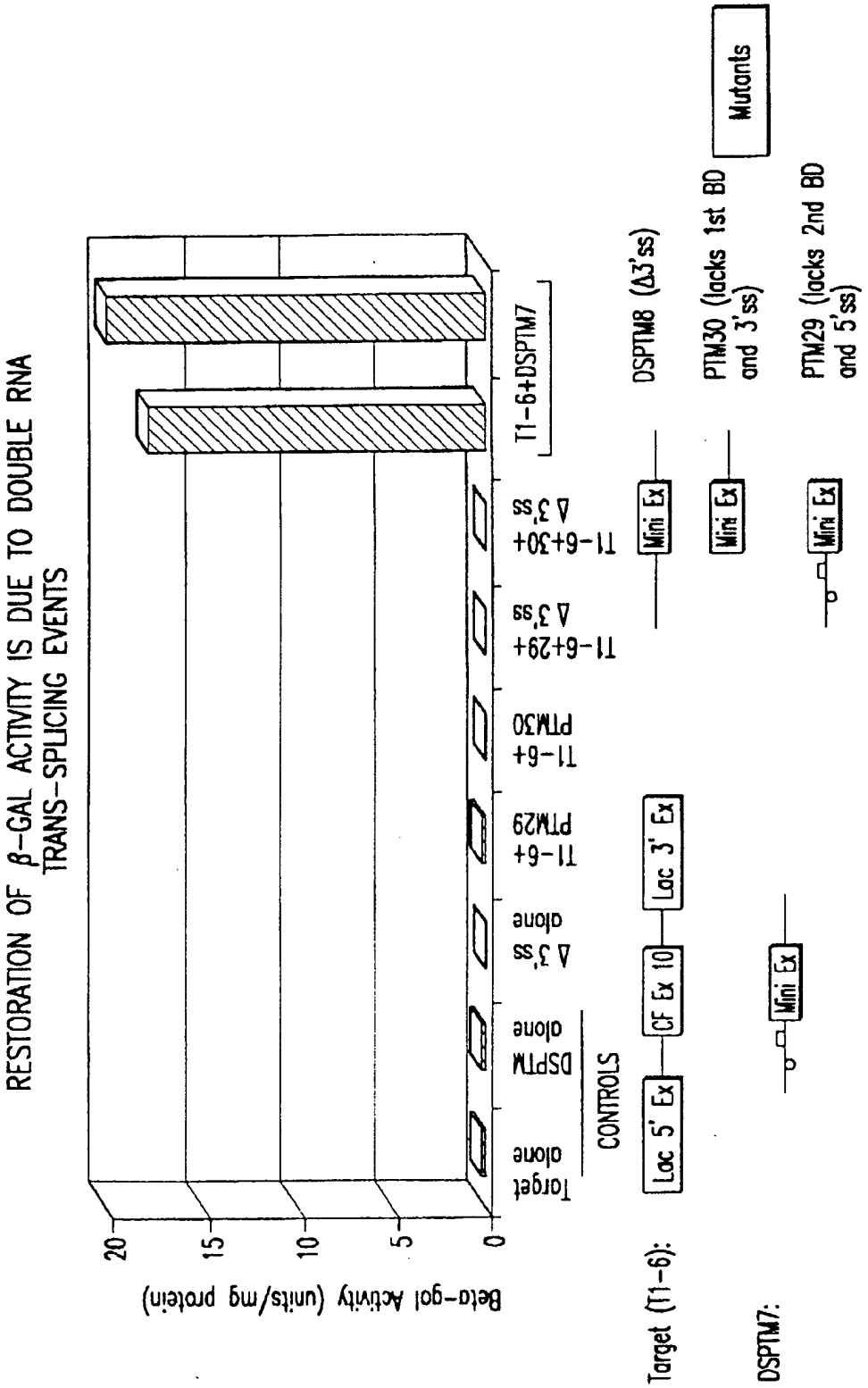

RESTORATION OF β-GAL ACTIVITY IS DUE TO DOUBLE RNA TRANS-SPLICING EVENTS
Beta-gal Activity (units/mg protein)
0
5
10
15
20
CONTROLS
Target alone
DSPTM alone
Δ 3'ss alone
T1-6+PTM29
T1-6+PTM30
T1-6+29+ Δ 3'ss
T1-6+30+ Δ 3'ss
T1-6+DSPTM7
DSPTM8 (Δ3'ss)
PTM30 (lacks 1st BD and 3'ss)
PTM29 (lacks 2nd BD and 5'ss)
Mutants
Target (T1-6):
Lac 5' Ex
CF Ex 10
Lac 3' Ex
Mini Ex
DSPTM7:
Mini Ex
Mini Ex
Mini Ex

FIG.26

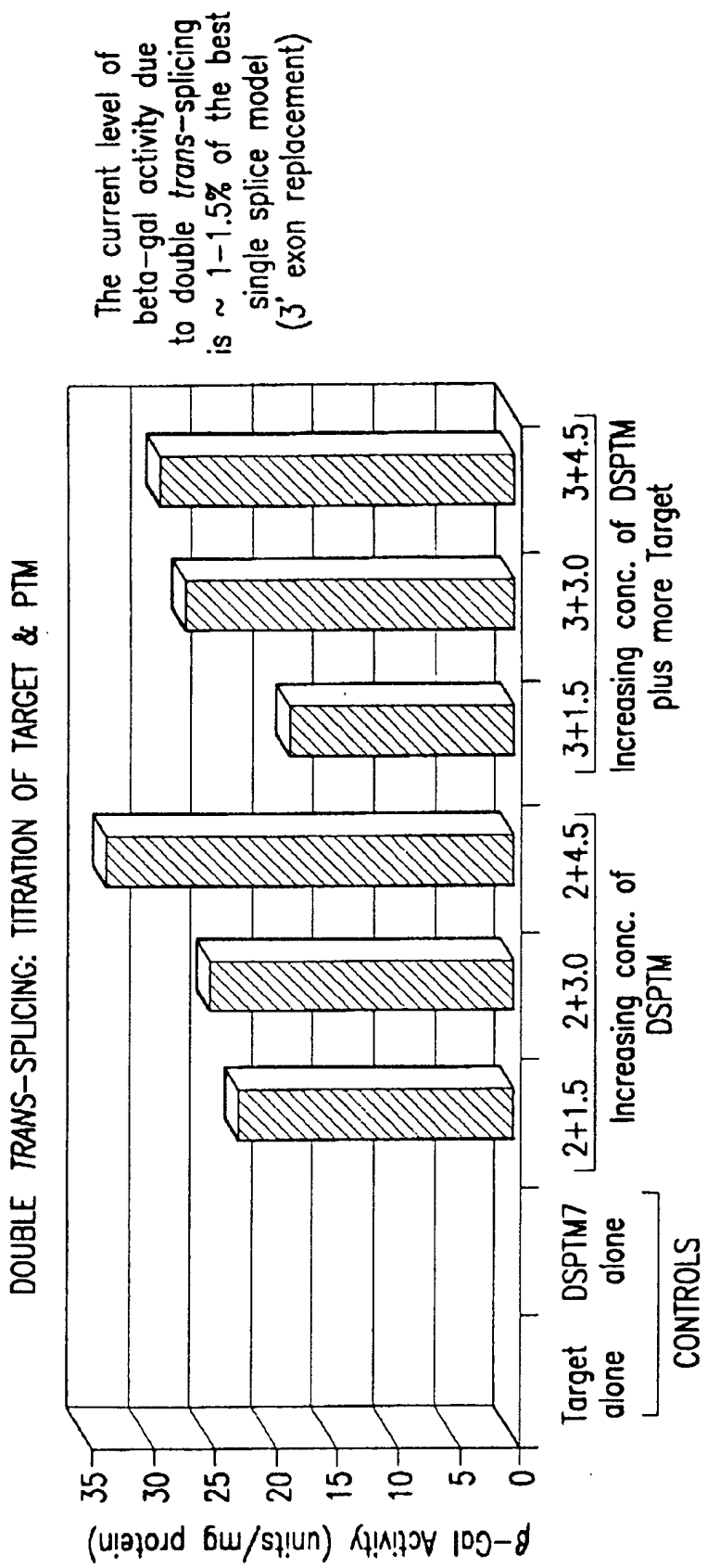

DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM
The current level of beta-gal activity due to double trans-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)
β-Gal Activity (units/mg protein)
0
5
10
15
20
25
30
35
Target alone
DSPTM7 alone
CONTROLS
2+1.5
2+3.0
2+4.5
Increasing conc. of DSPTM
3+1.5
3+3.0
3+4.5
Increasing conc. of DSPTM plus more Target

FIG.27

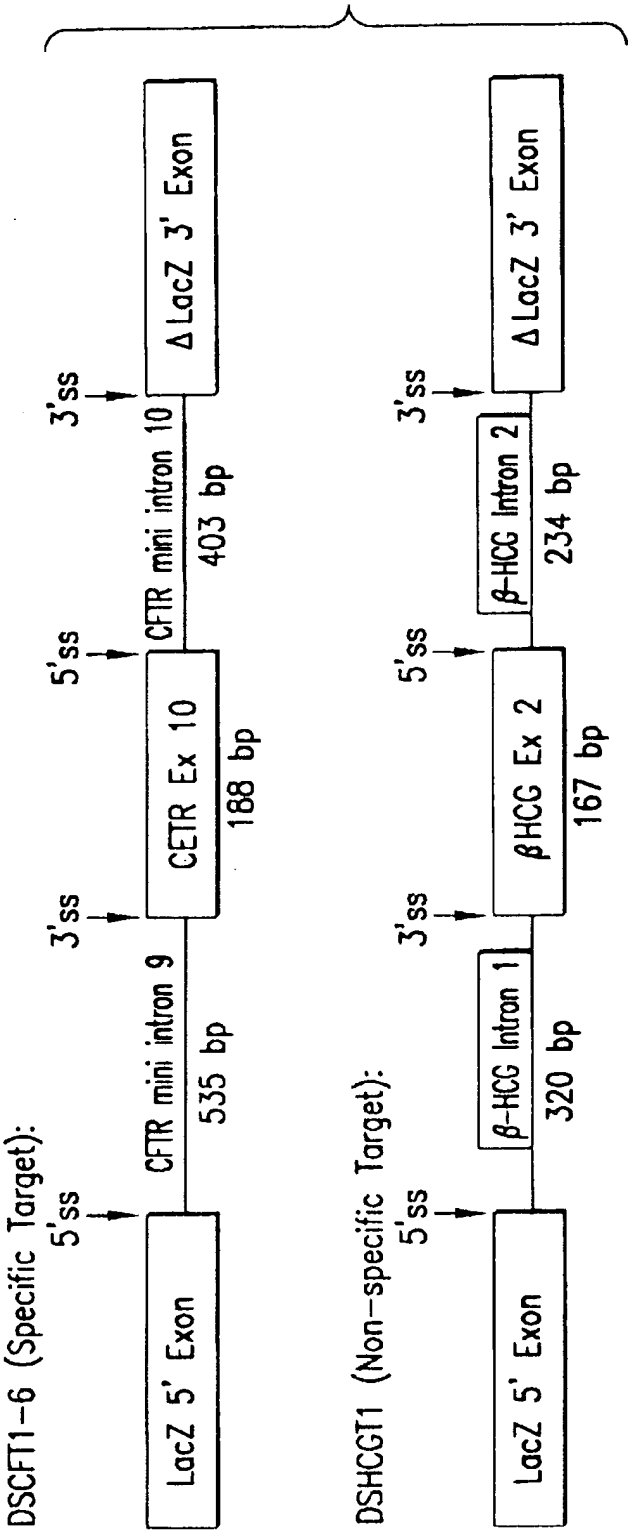

DSCFT1-6 (Specific Target):
5'ss
LacZ 5' Exon
CFTR mini intron 9
535 bp
3'ss
CETR Ex 10
188 bp
5'ss
CFTR mini intron 10
403 bp
3'ss
Δ LacZ 3' Exon
DSHCGT1 (Non-specific Target):
5'ss
LacZ 5' Exon
β-HCG Intron 1
320 bp
3'ss
βHCG Ex 2
167 bp
5'ss
β-HCG Intron 2
234 bp
3'ss
Δ LacZ 3' Exon

FIG.28

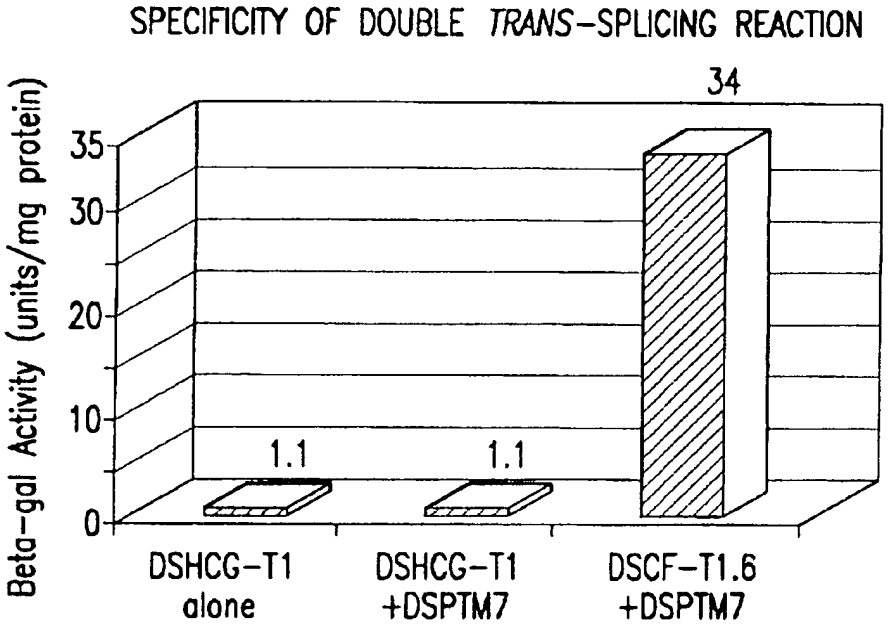

SPECIFICITY OF DOUBLE *TRANS*-SPLICING REACTION
Beta-gal Activity (units/mg protein)
35
30
20
10
0
34
1.1
1.1
DSHCG-T1 alone
DSHCG-T1 +DSPTM7
DSCF-T1.6 +DSPTM7

FIG.29

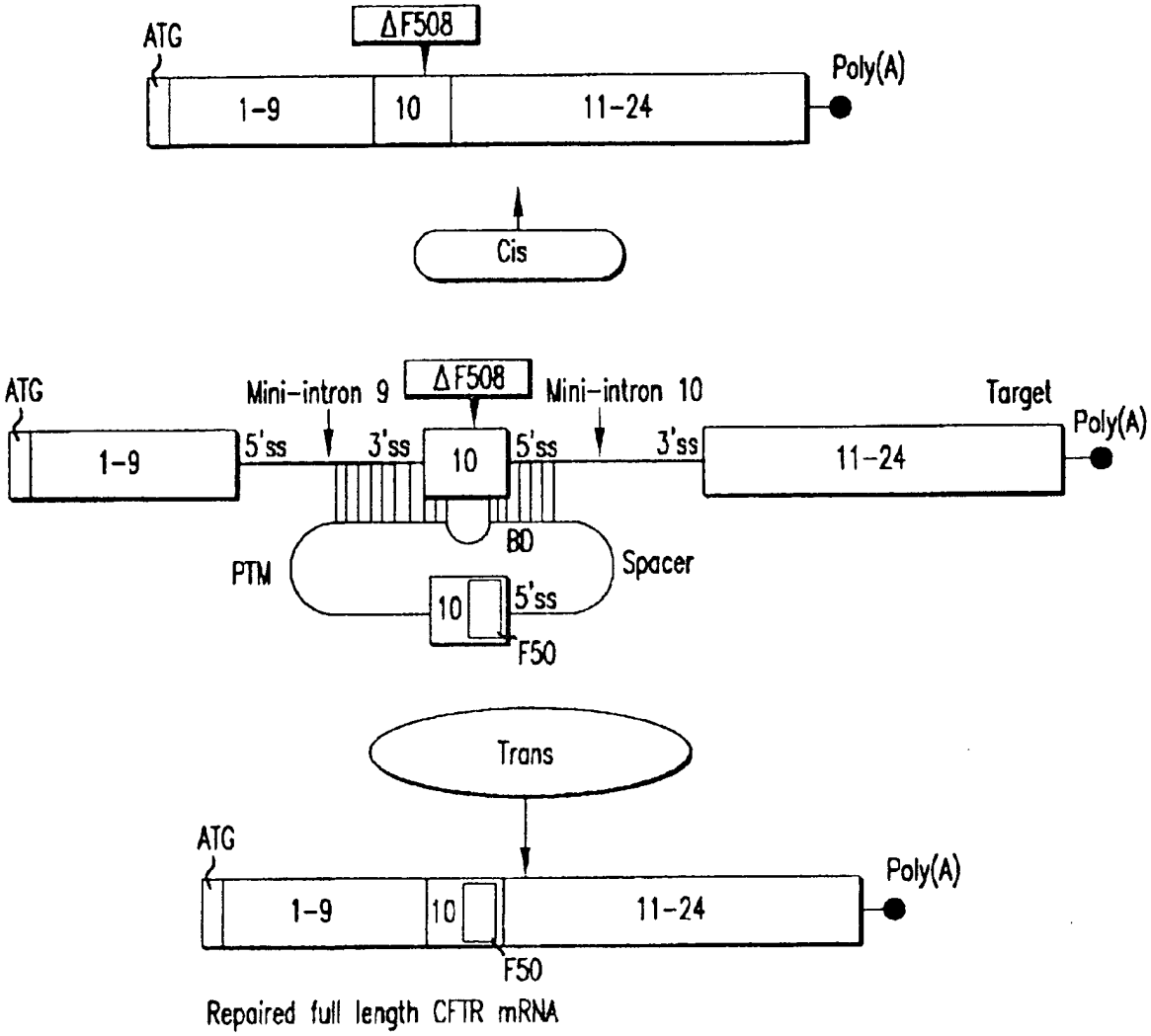
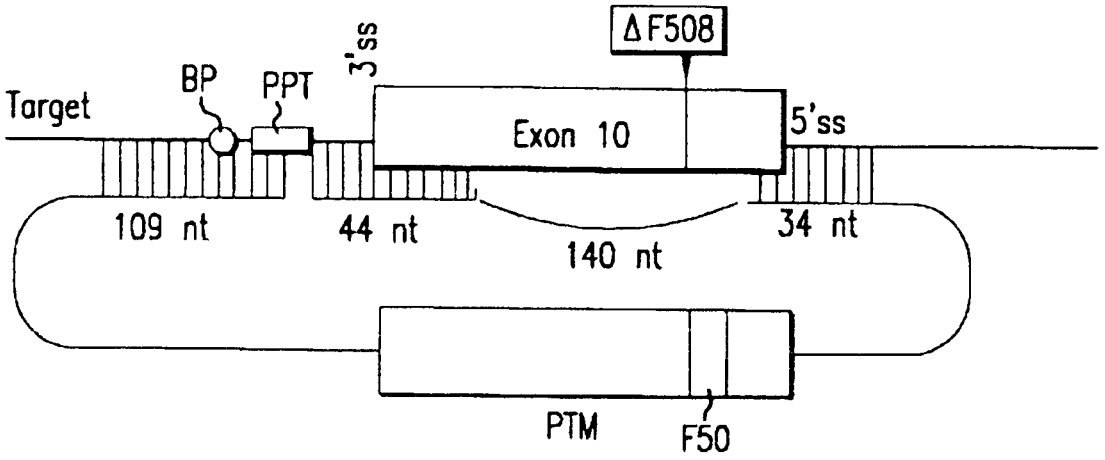

ATG
ΔF508
Poly(A)
1-9
10
11-24
Cis
ATG
Mini-intron 9
ΔF508
Mini-intron 10
Target
Poly(A)
1-9
5'ss
3'ss
10
5'ss
3'ss
11-24
BD
PTM
Spacer
10
5'ss
F50
Trans
ATG
Poly(A)
1-9
10
11-24
F50
Repaired full length CFTR mRNA

FIG.30

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGCTCATGATGATCATGGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCG
GCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT
C*TT*CGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGAGGAG

MCU in exon 10 of PTM
88 OF 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

FIG.31

Sequence of a double
Trans–spliced product

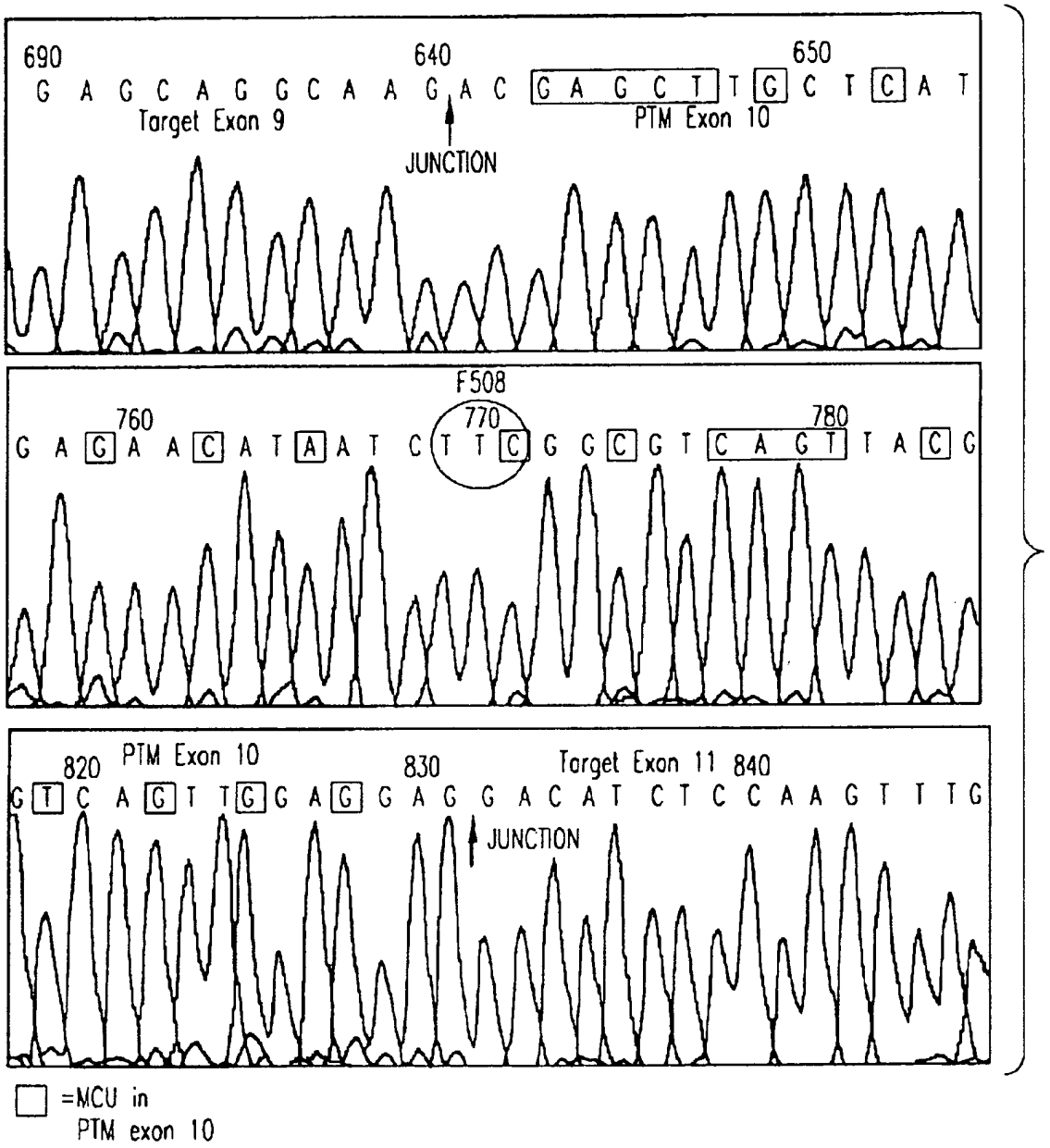


FIG.32

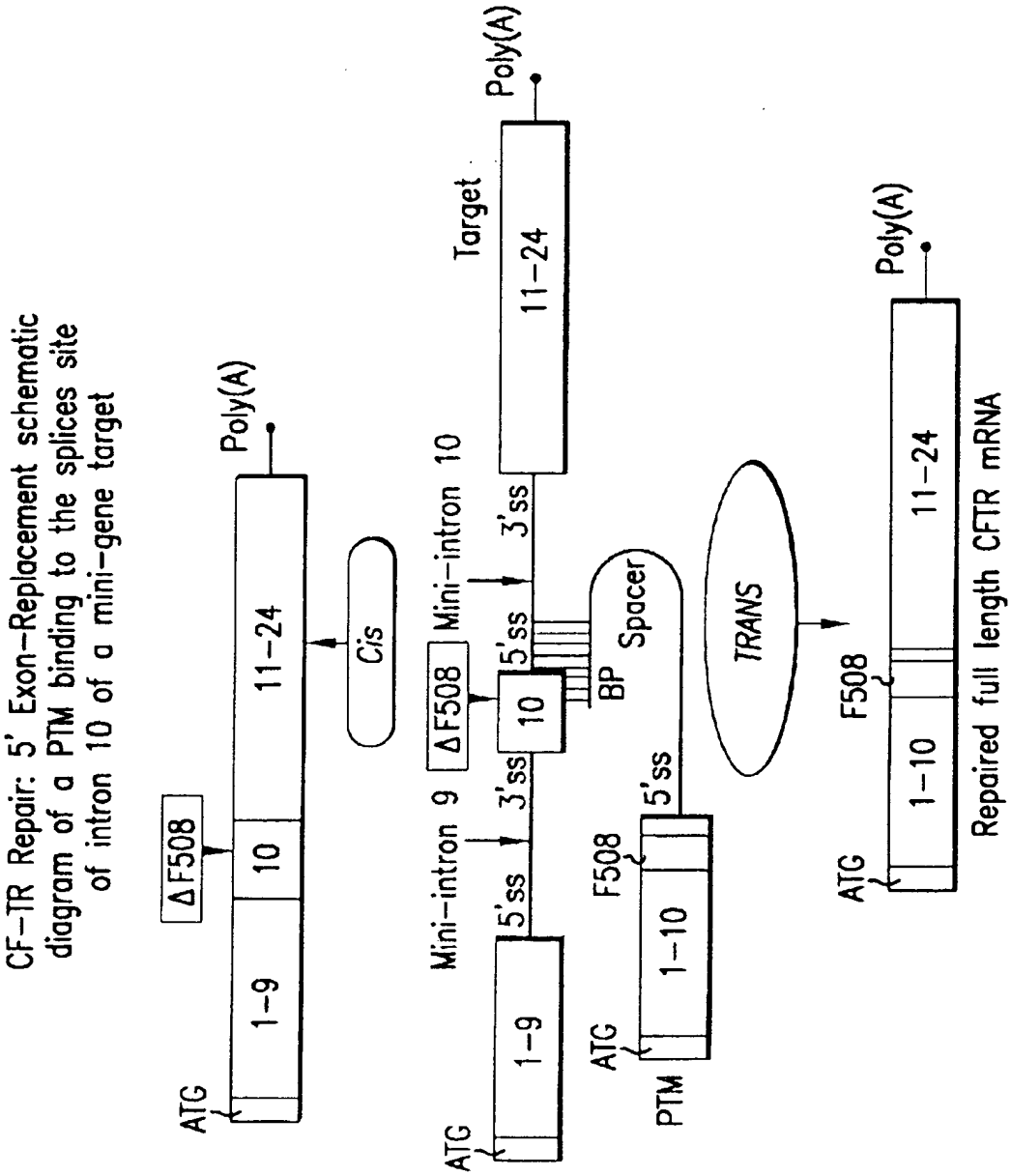

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splices site of intron 10 of a mini-gene target
ATG
1-9
ΔF508
10
11-24
Poly(A)
Cis
ATG
1-9
Mini-intron 9
5'ss
3'ss
ΔF508
10
5'ss
Mini-intron 10
3'ss
Target
11-24
Poly(A)
BP
Spacer
ATG
F508
1-10
5'ss
PTM
TRANS
ATG
1-10
F508
11-24
Poly(A)
Repaired full length CFTR mRNA

FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

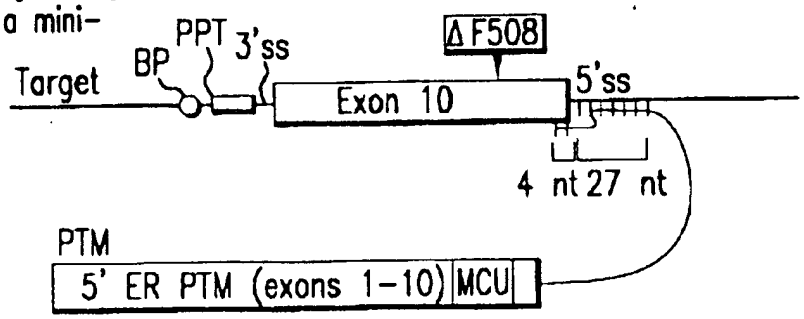


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

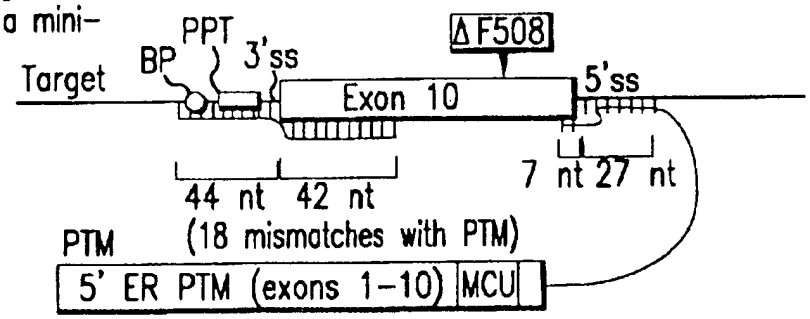


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

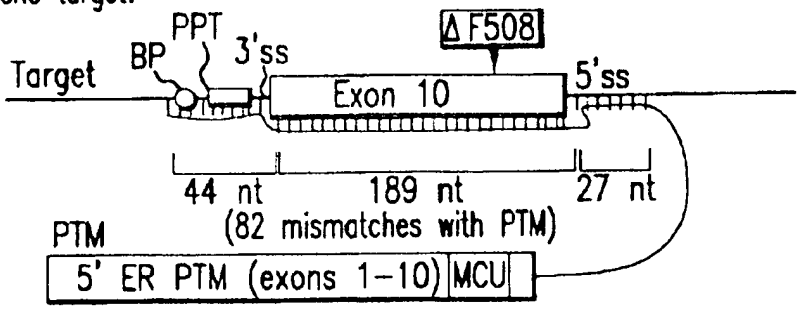
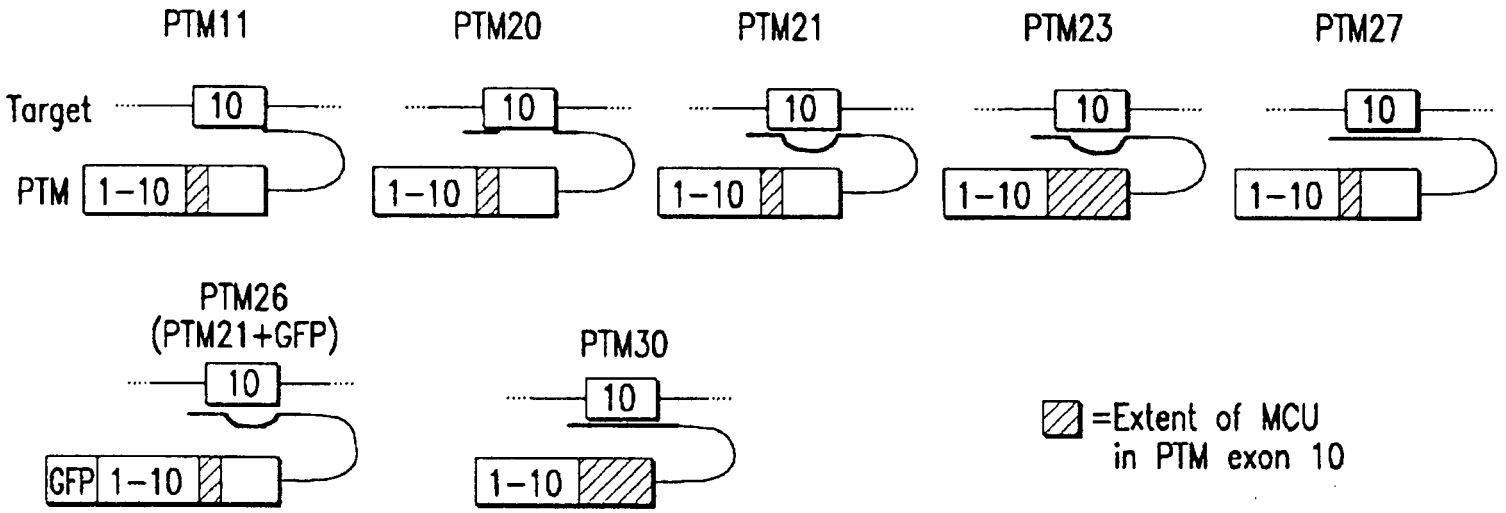


FIG.34C



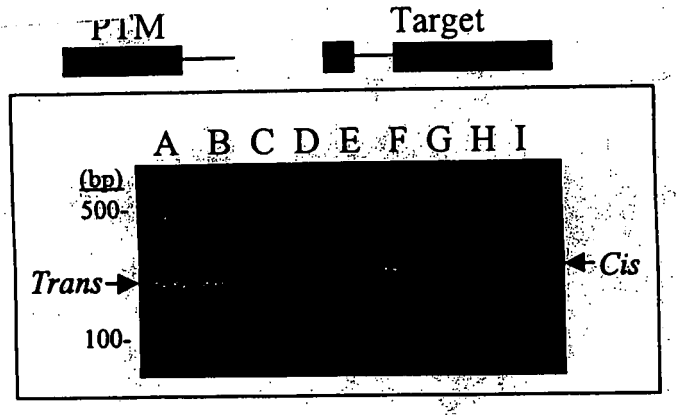
MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complemetary to its binding domain.

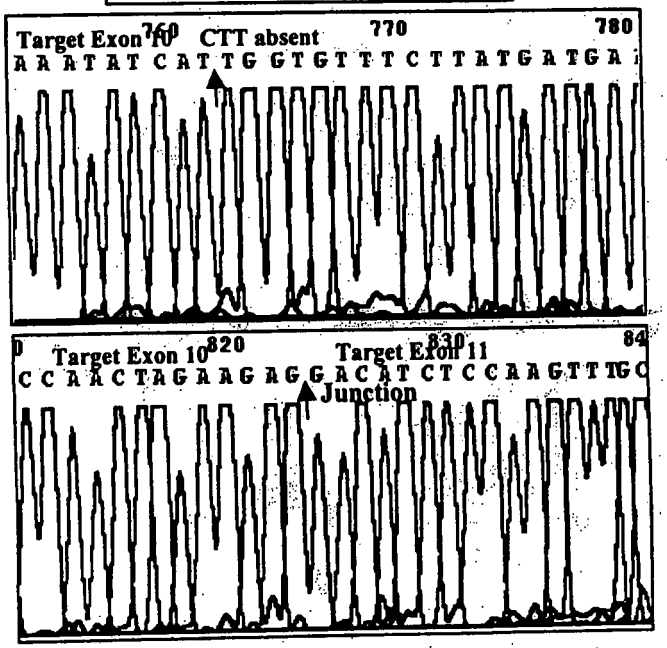
ACGAGCTTGCTCATGATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCG
GCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGAGGAG

FIG.35

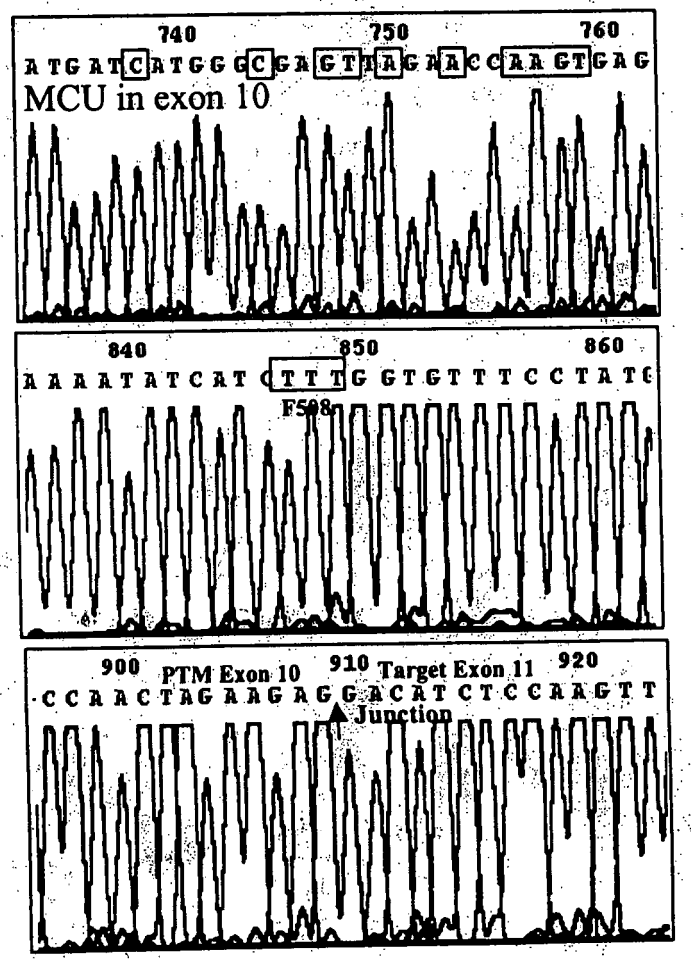
Figure 36



A. *Cis*-spliced product
[Primers CF1 + CF111]



B. *Trans*-spliced product
[Primers CF93 + CF111]



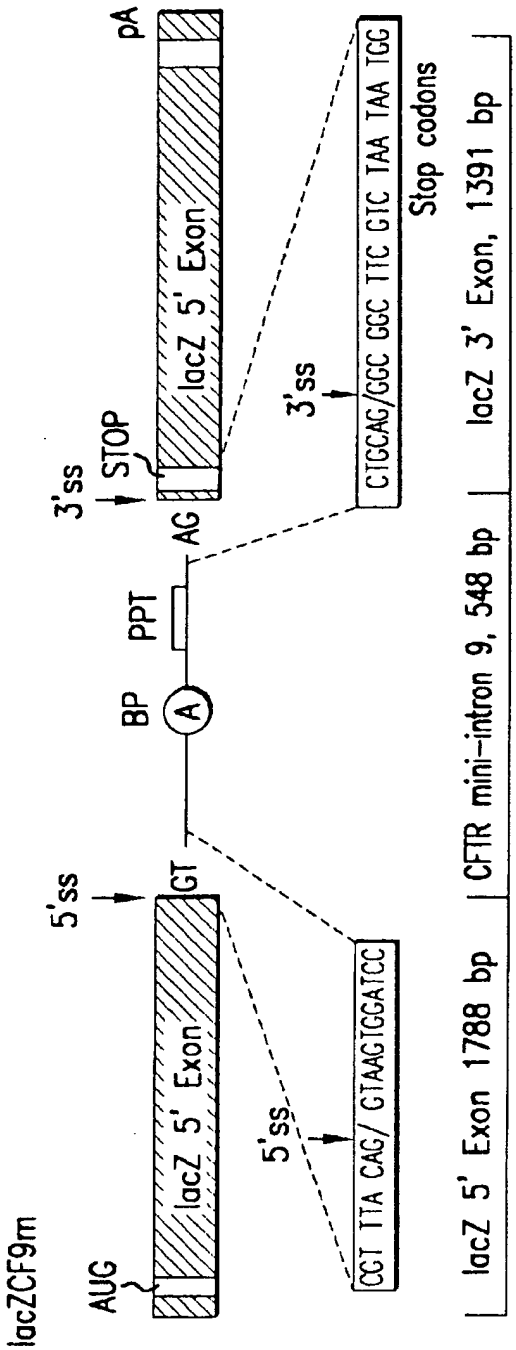

lacZCF9m
AUG
lacZ 5' Exon
5'ss
GT
BP
A
PPT
AG
3'ss
STOP
pA
lacZ 5' Exon
5'ss
CCT TTA CAG/ GTAAGTGGATCC
3'ss
CTGCAG/GGC GGC TTC GTC TAA TAA TGG
Stop codons
lacZ 5' Exon 1788 bp
CFTR mini-intron 9, 548 bp
lacZ 3' Exon, 1391 bp

FIG.37A

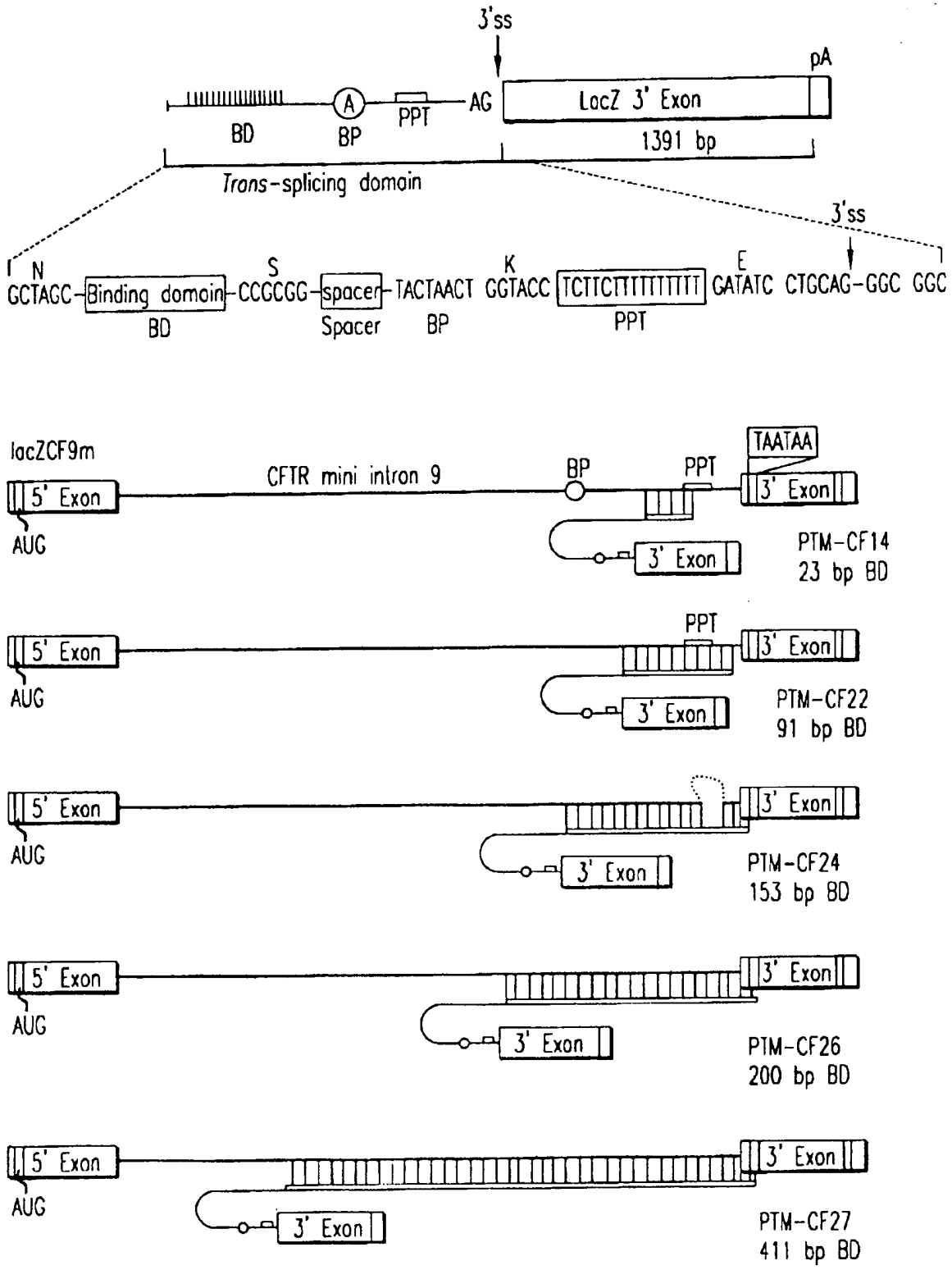

3'ss
pA
BD
BP
PPT
AG
LacZ 3' Exon
1391 bp
Trans-splicing domain
3'ss
N
S
K
E
GCTAGC-Binding domain-CCGCGG-spacer-TACTAACT GGTACC TCTTCTTTTTTTTT GATATC CTGCAG-GGC GGC
BD
Spacer
BP
PPT
lacZCF9m
5' Exon
AUG
CFTR mini intron 9
BP
PPT
TAATAA
3' Exon
PTM-CF14
23 bp BD
PTM-CF22
91 bp BD
PTM-CF24
153 bp BD
PTM-CF26
200 bp BD
PTM-CF27
411 bp BD

FIG.37B

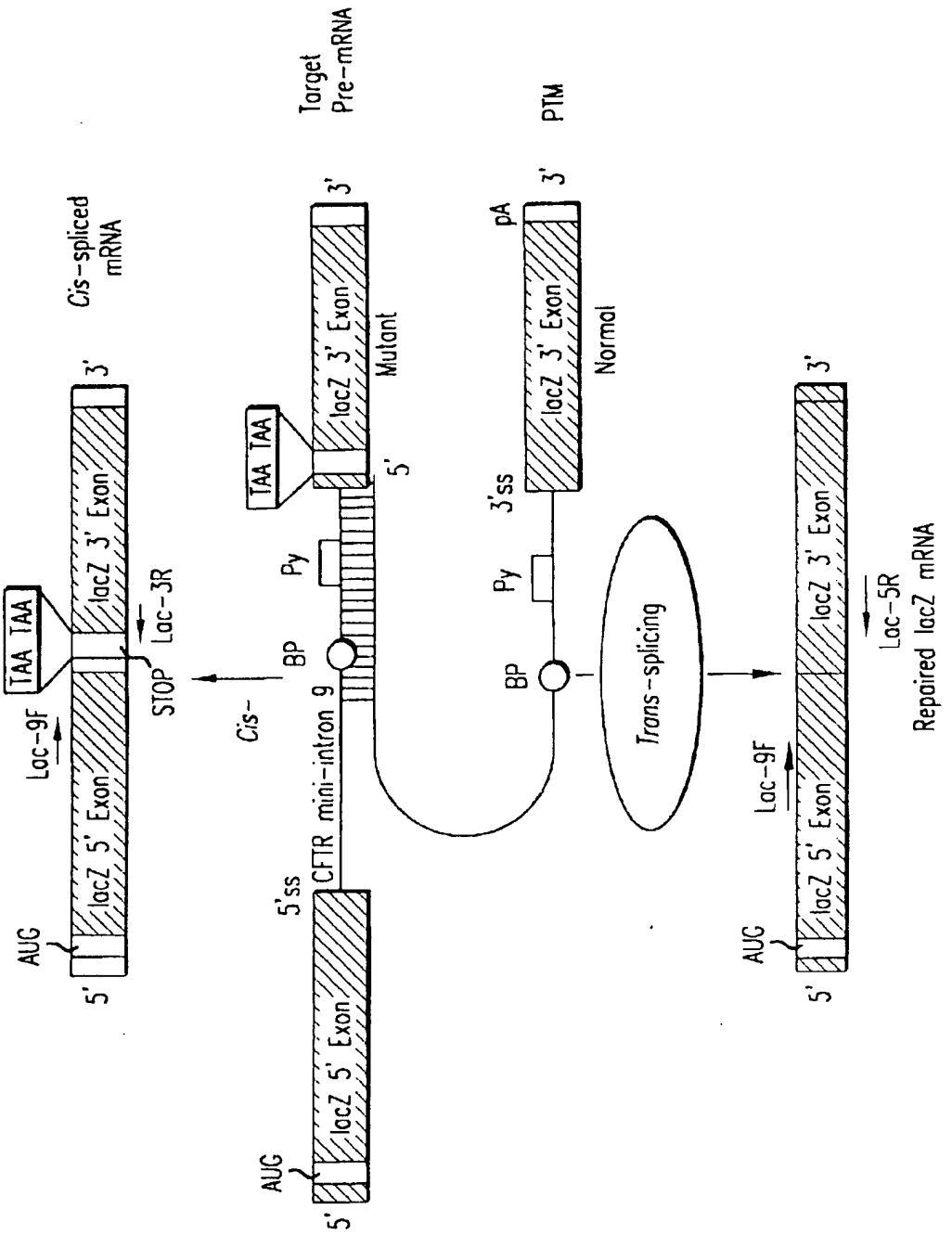

Cis-spliced mRNA
Target Pre-mRNA
PTM
AUG
5'
lacZ 5' Exon
Loc-9F
TAA TAA
lacZ 3' Exon
3'
Lac-3R
STOP
Cis-
BP
Py
5'ss
CFTR mini-intron 9
3'ss
pA
Mutant
Normal
Trans-splicing
Lac-5R
Repaired lacZ mRNA

FIG.37C

A

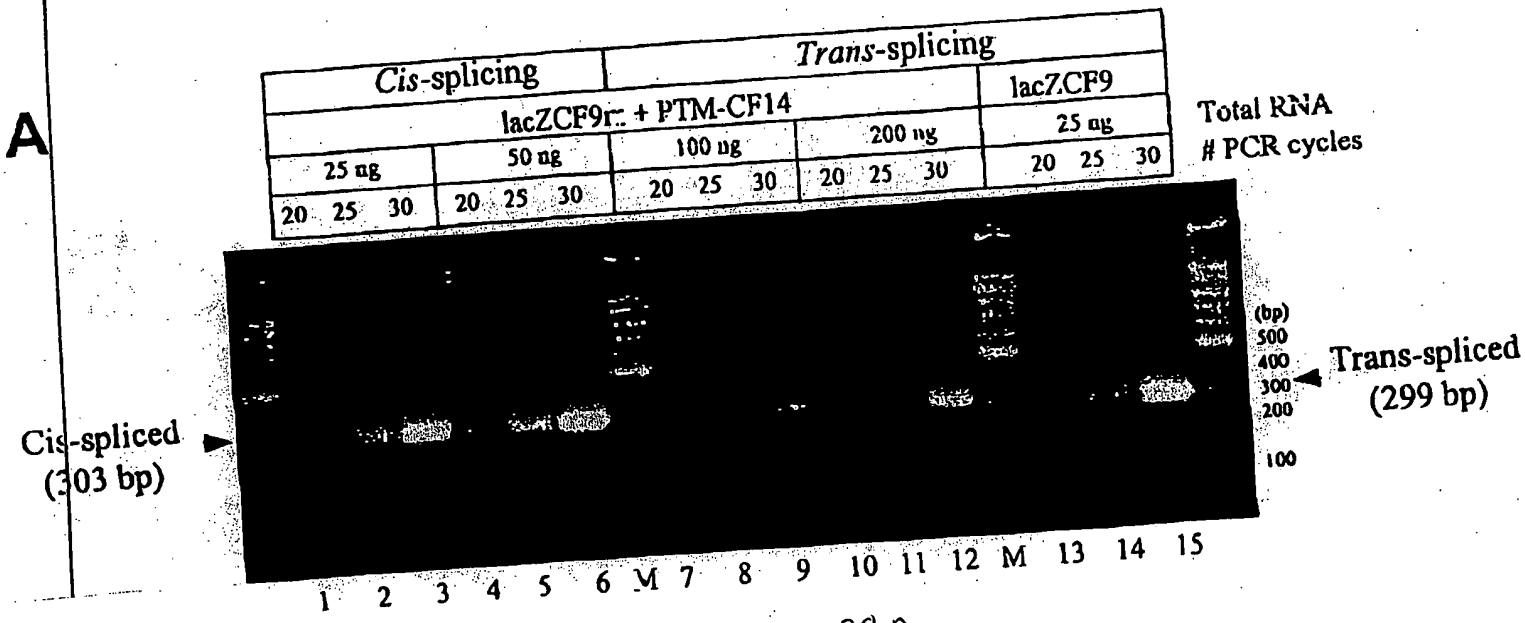
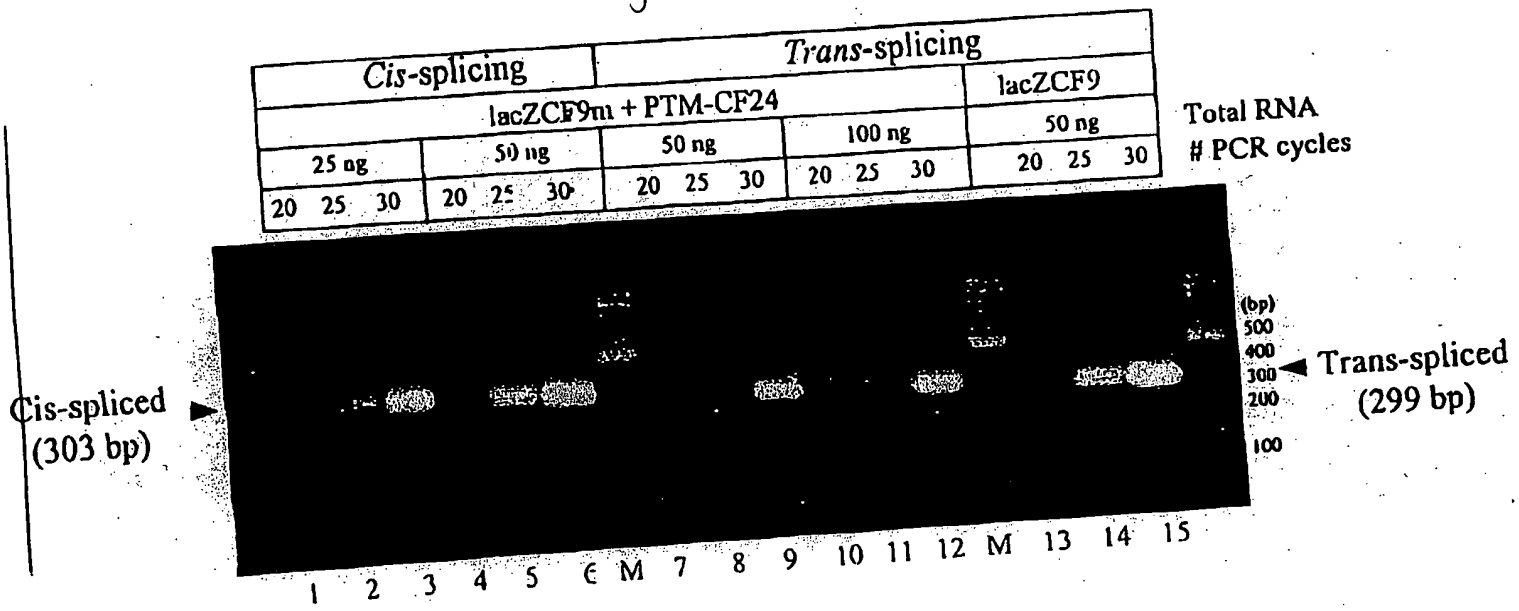

Cis-splicing
Trans-splicing
lacZCF9m + PTM-CF14
lacZCF9
25 ng
50 ng
100 ng
200 ng
25 ng
20 25 30
Total RNA
PCR cycles
(bp)
500
400
300
200
100
Cis-spliced
(303 bp)
Trans-spliced
(299 bp)
1 2 3 4 5 6 M 7 8 9 10 11 12 M 13 14 15

Figure 38A


Cis-splicing
Trans-splicing
lacZCF9m + PTM-CF24
lacZCF9
25 ng
50 ng
50 ng
100 ng
50 ng
20 25 30
Total RNA
PCR cycles
(bp)
500
400
300
200
100
Cis-spliced
(303 bp)
Trans-spliced
(299 bp)
1 2 3 4 5 6 M 7 8 9 10 11 12 M 13 14 15

B

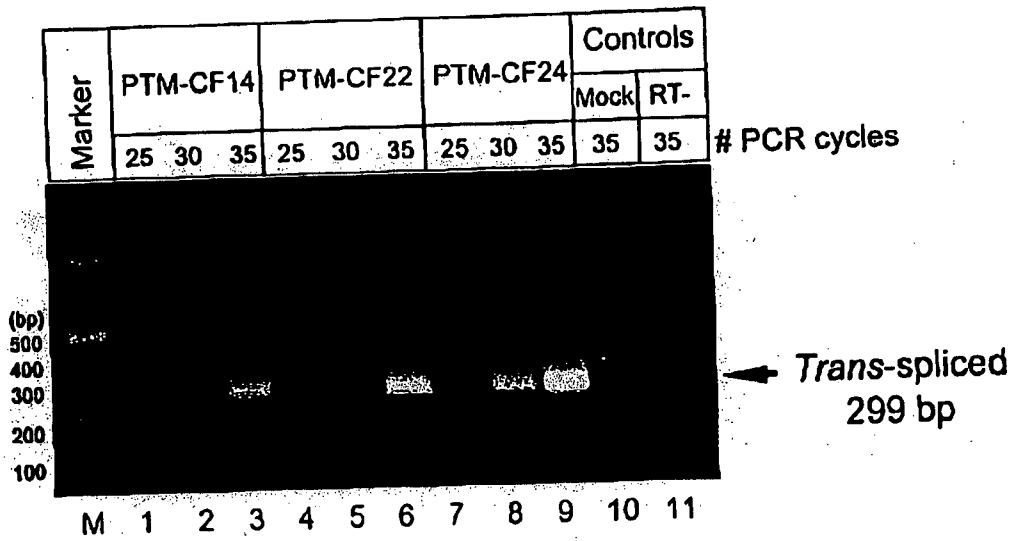

Marker
PTM-CF14
PTM-CF22
PTM-CF24
Controls
Mock
RT-
25 30 35 25 30 35 25 30 35 35 35
PCR cycles
(bp)
500
400
300
200
100
Trans-spliced
299 bp
M 1 2 3 4 5 6 7 8 9 10 11

Figure 38B

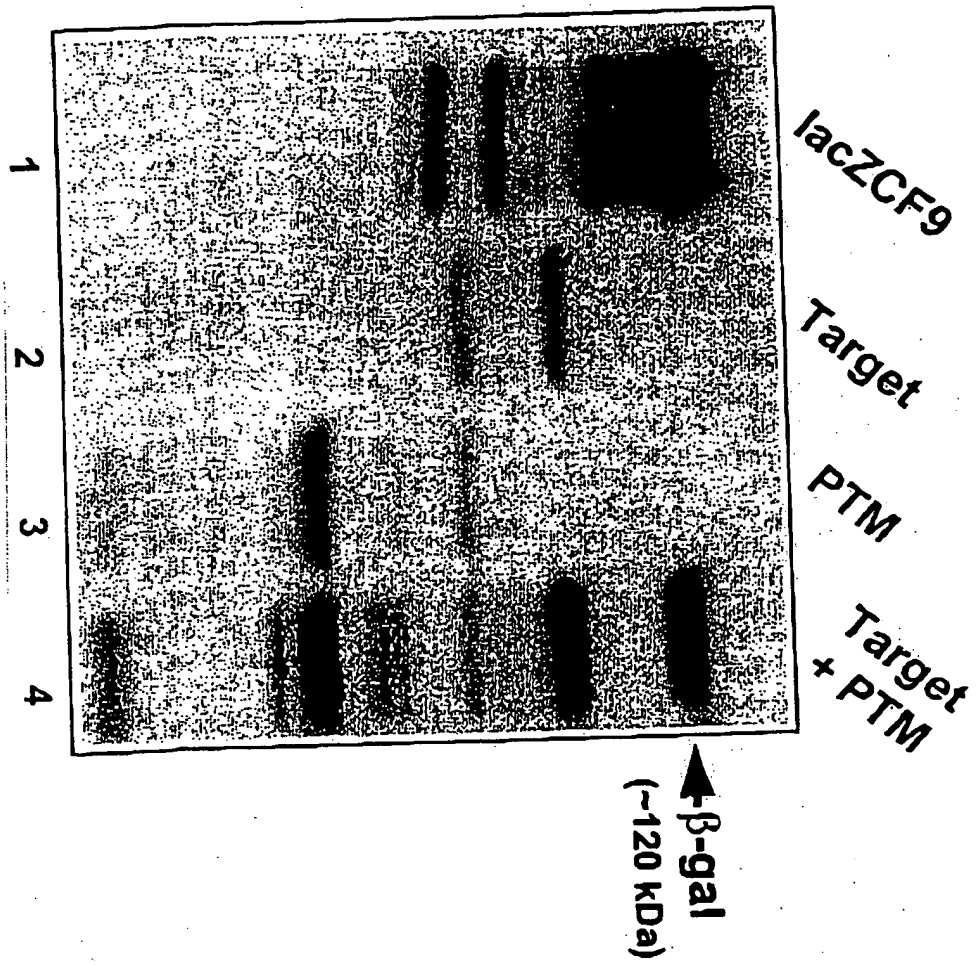


Figure 39

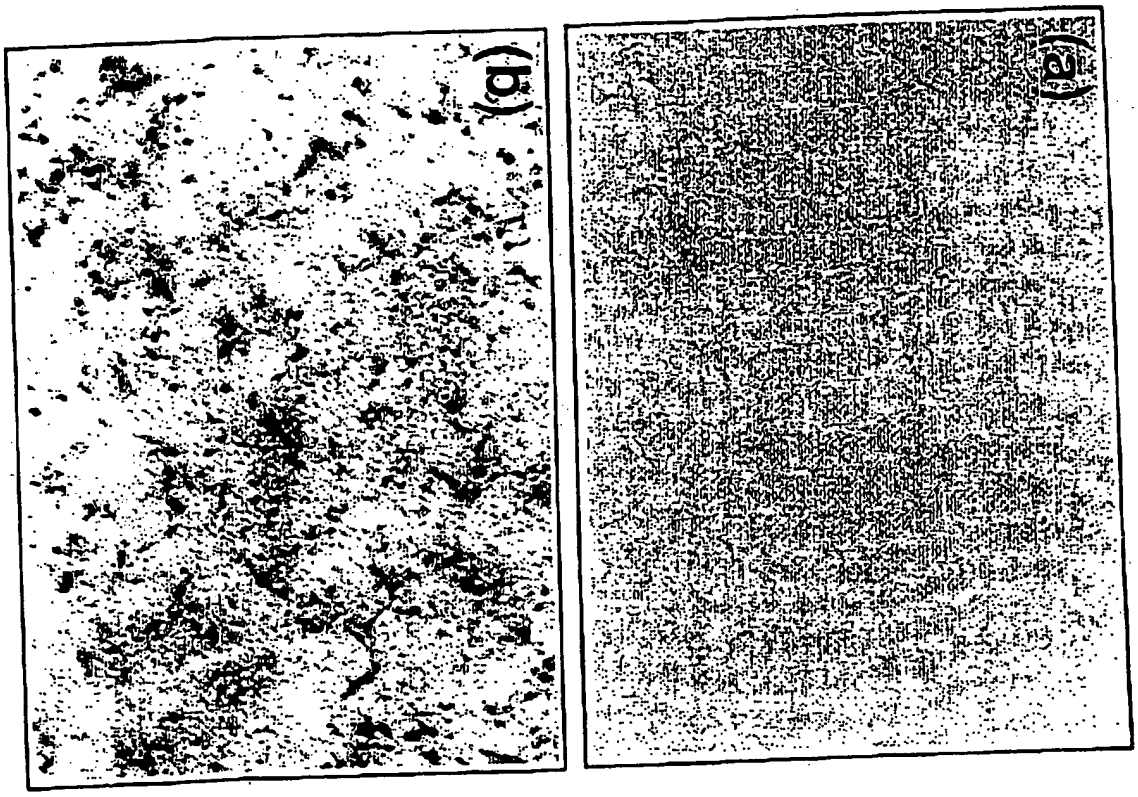


Figure 40A

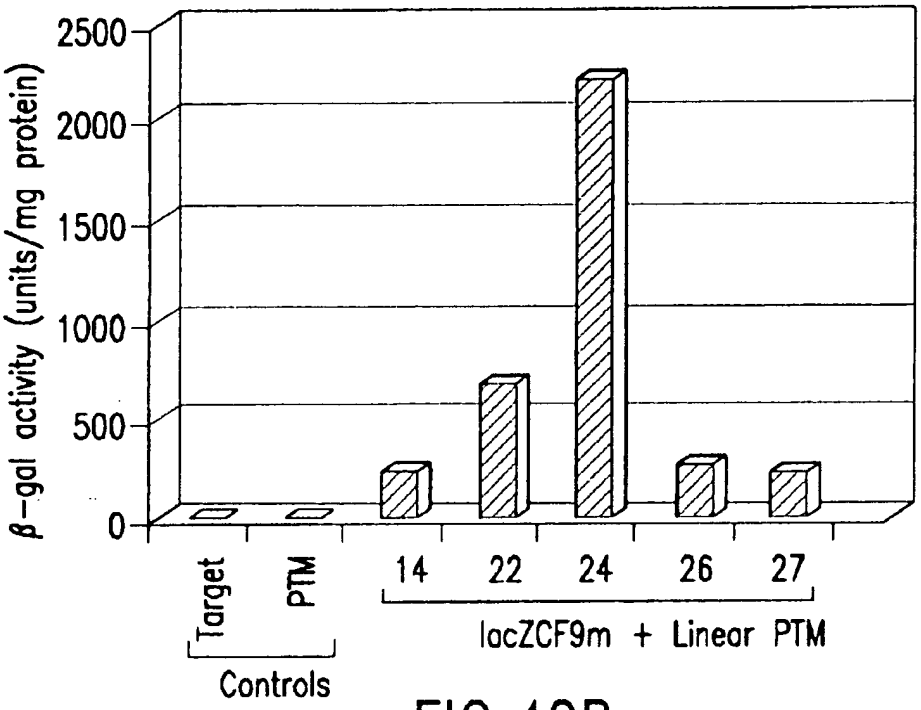


FIG.40B

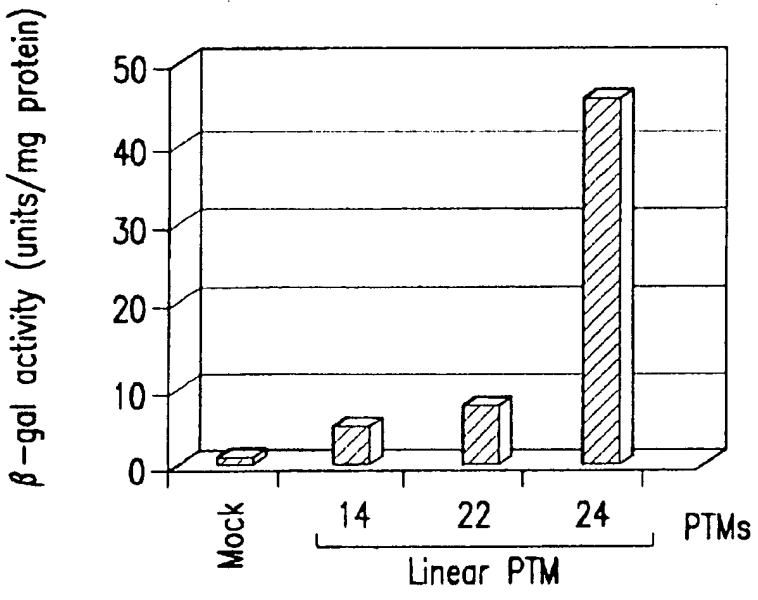


FIG.40C

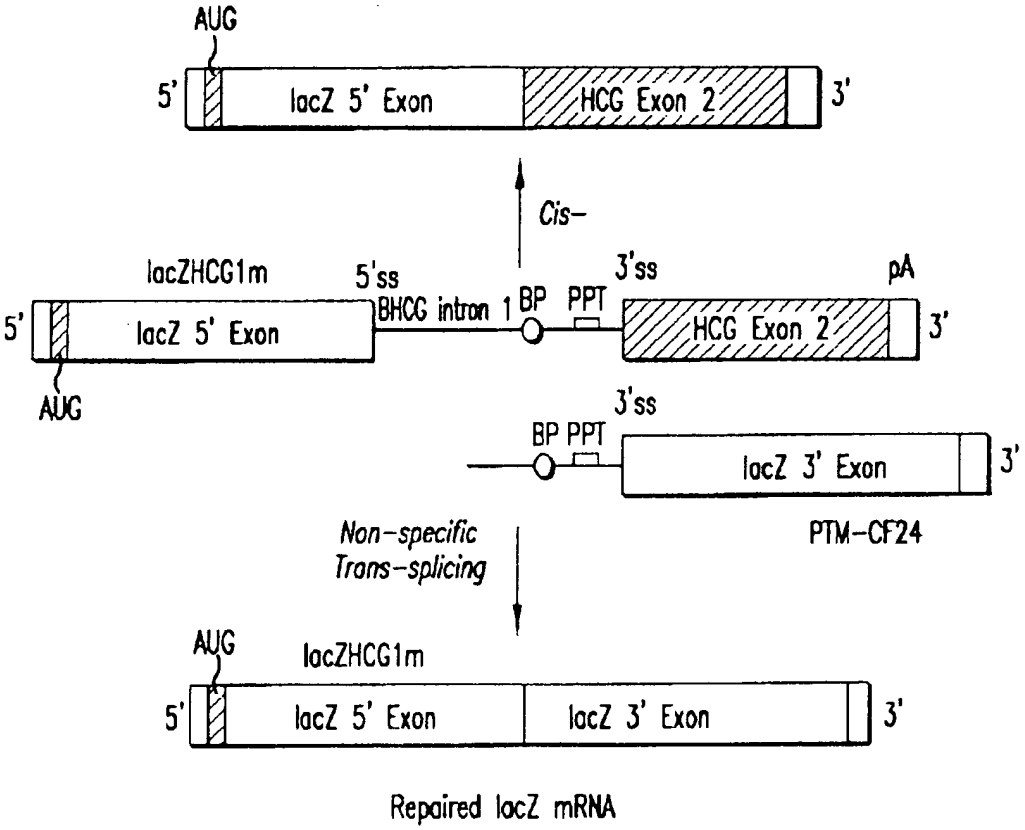

AUG
5'
lacZ 5' Exon
HCG Exon 2
3'
Cis-
lacZHCG1m
5'ss
3'ss
pA
5'
lacZ 5' Exon
BHCG intron 1
BP
PPT
HCG Exon 2
3'
AUG
3'ss
BP PPT
lacZ 3' Exon
3'
Non-specific
Trans-splicing
PTM-CF24
AUG
lacZHCG1m
5'
lacZ 5' Exon
lacZ 3' Exon
3'
Repaired lacZ mRNA

FIG.41A

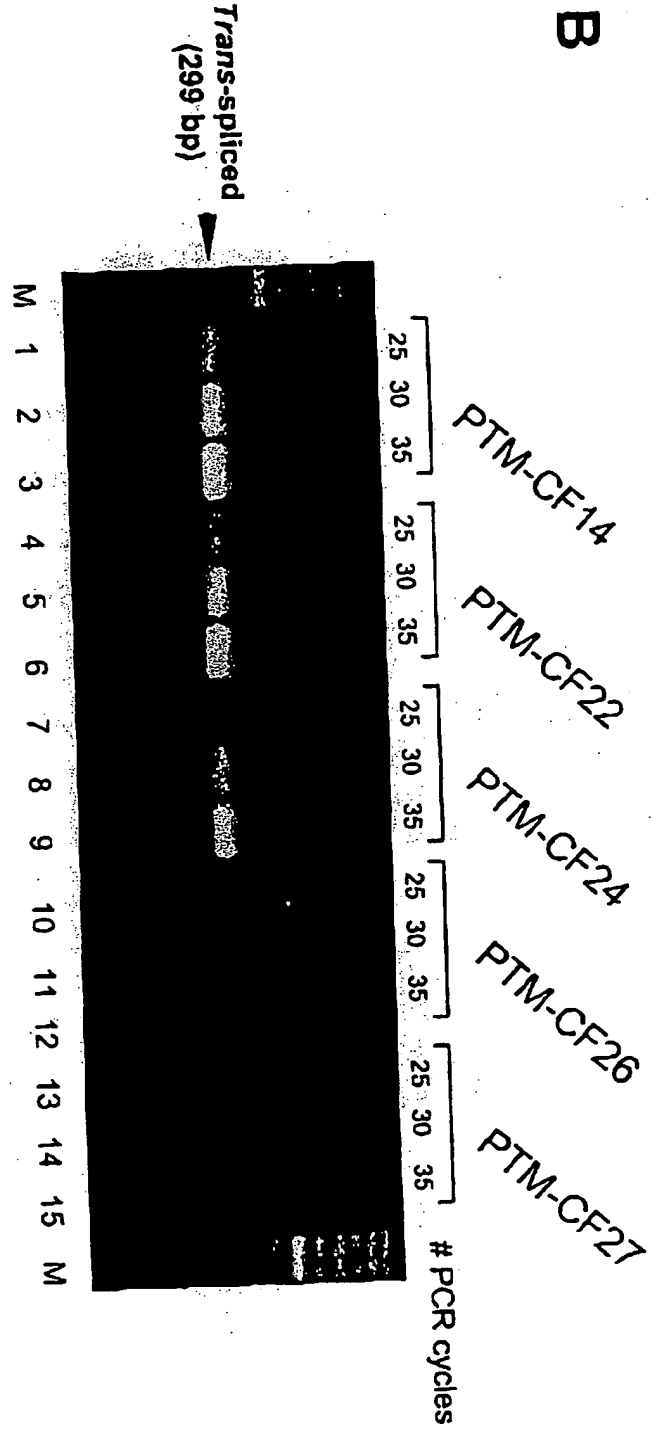

B
Trans-spliced
(299 bp)
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 M
25 30 35
PTM-CF14
25 30 35
PTM-CF22
25 30 35
PTM-CF24
25 30 35
PTM-CF26
25 30 35
PTM-CF27
PCR cycles

Figure 4B

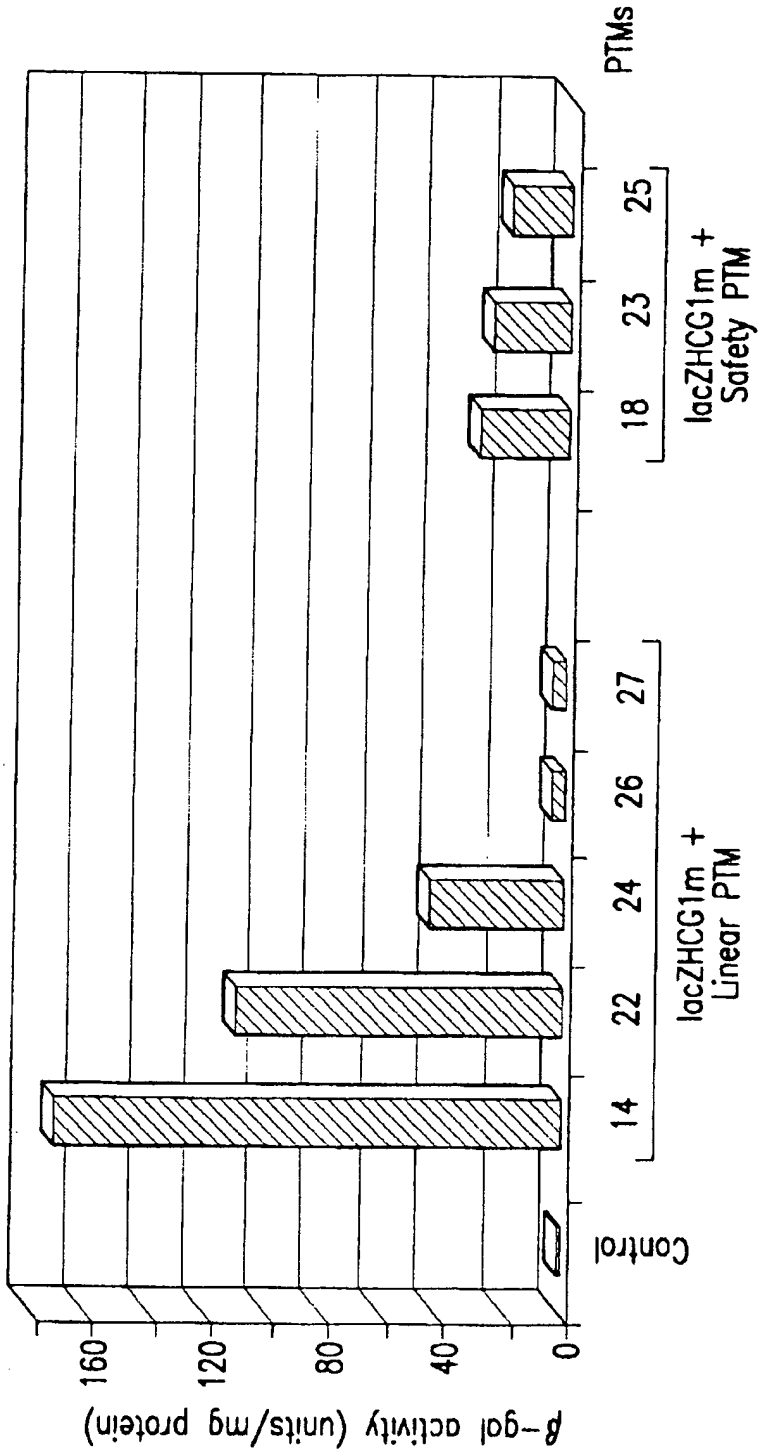

β-gal activity (units/mg protein)
0
40
80
120
160
Control
14
22
24
26
27
lacZHCG1m + Linear PTM
18
23
25
lacZHCG1m + Safety PTM
PTMs

FIG.41C

Exons
1-10
ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
GATACAGACAGCGCCTGGAATTGTCAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTTTTCTGG
AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA
TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT
TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT
TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTCCTTT
CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAG
GCTGGGCTAGGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAAACTTAAGACA
AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTT
GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTCACCACCATCTCATTCT
GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
AATGTAACAGCCTTCTGGGAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT
CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGAAAGATATTAATTTCAAGAT
AGAAAGAGGACAGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGGCGAG
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGA
GGAG
Trans-splicing domain
GTAAGATATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGT
GCTTAATTTTACCCTCTGAATTCTCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATT
ATTAACTCATTATCAAATCACGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I

GCTAGC-AATAATGACGAAGCCGCCCCTCACGCTCAGGATTCACTTGCCTCCAATTATCATCCTAAGCAGAAGTGTATA

153 bp BD underlined

TTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGC

Sac II

AC-CCGCGG

FIG.43A

Trans-splicing domain

AATAATGACGAAGCCGCCCCTCACGCTCAGGATTCACTTGCCCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTA
TTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTCGAATACTAACTGGTACCTCTTCTTTTTTTTTTGATATCCTGCAG

Exons10-24

ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAAGAATTTCATTCT
GTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAATATAGATA
CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA
GGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTGTATT
TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGC
TAACAAAACTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT
AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATT
CTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGTTTCTCATTAGAAGGAGATGC
TCCTGTCTCCTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAAGAATTCTATT
CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT
CTGATGAGCCTTTAGAGAGAAGGCTGTCCTTAGTACCAGATTCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT
GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTGAACCTGATGACACACTCAGTTAACCAAGGT
CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGAACTGGATA
TATATTCAAGAAGGTTATCTCAAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA
ATTTTTGTGCTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTGCTGTGGCTCCTTGGAA
ACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC
GTATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG
CATACTCTAATCACAGTGTCGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCCTCA
ACACGTTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT
ATTTGACTTCATCCAGTTGTTATTAATTGTGATTGGAGCTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTTGTT
GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCCTCCAAACCTCACAGCAACTCAAACAACTGG
AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTCGGACG
GCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTG
CGCTGGTTCCAAATGAGAATAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAACAACAG
GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGGCTGTAAACTC
CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGTAAACCT
ACCAAGTCAACCAAACCATACAAGAATGGCCAACTCTCGAAAGTTATGATTATTGAGAATTCACACGTGAAGAAAGATG
ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA
GAACATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGGCCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA
TCAGCTTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGCAAC
AGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCTGGAACATTTAGAAAAAACTTGGATCCCTA
TGAACAGTGGAGTGATCAAGAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCCTGGG
AAGCTTGACTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
TTCTCAGTAAGGCGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAAATAATTAGAAG
AACTCTAAAACAAGCATTTGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
TTTTTGGTCATAGAAGAGAACAAAGTGCGGCAGTACGATTCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTTCCGGC
AAGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTCCCCACCGGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGC

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATTAG

FIG.43B